

Research article

Cellulose acetate phthalate, a common pharmaceutical excipient, inactivates HIV-1 and blocks the coreceptor binding site on the virus envelope glycoprotein gp120

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Published: 25 September 2001

Received: 3 July 2001

BMC Infectious Diseases 2001, 1:17

Accepted: 25 September 2001

This article is available from: <http://www.biomedcentral.com/1471-2334/1/17>

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Abstract

Background: Cellulose acetate phthalate (CAP), a pharmaceutical excipient used for enteric film coating of capsules and tablets, was shown to inhibit infection by the human immunodeficiency virus type 1 (HIV-1) and several herpesviruses. CAP formulations inactivated HIV-1, herpesvirus types 1 (HSV-1) and 2 (HSV-2) and the major nonviral sexually transmitted disease (STD) pathogens and were effective in animal models for vaginal infection by HSV-2 and simian immunodeficiency virus.

Methods: Enzyme-linked immunoassays and flow cytometry were used to demonstrate CAP binding to HIV-1 and to define the binding site on the virus envelope.

Results: 1) CAP binds to HIV-1 virus particles and to the envelope glycoprotein gp120; 2) this leads to blockade of the gp120 V3 loop and other gp120 sites resulting in diminished reactivity with HIV-1 coreceptors CXCR4 and CCR5; 3) CAP binding to HIV-1 virions impairs their infectivity; 4) these findings apply to both HIV-1 IIIB, an X4 virus, and HIV-1 BaL, an R5 virus.

Conclusions: These results provide support for consideration of CAP as a topical microbicide of choice for prevention of STDs, including HIV-1 infection.

Background

Due to the current unavailability of anti-HIV vaccines, other preventive methods have to be developed to control the ongoing AIDS pandemic. This includes the design and application of safe and effective topical microbicides. Screening of pharmaceutical excipients revealed that cellulose acetate phthalate (CAP), commonly used for enteric coating of tablets and capsules [1], has anti-HIV-1 activity. CAP in micronized form and formulated into a cream, is a broad spectrum microbicide inac-

tivating several sexually transmitted disease (STD) pathogens [2–4], including HIV-1 [2,5]. It was of interest to explore the mechanism(s) whereby CAP causes inactivation of HIV-1. Since CAP has a relatively high molecular weight ($M_w \sim 60,000$; [2]), its effect on HIV-1 virions would be expected to be confined to the virus surface, i.e. to the envelope glycoproteins gp120 and/or gp41. Thus, CAP would be expected to affect one or more steps required for HIV-1 entry into cells, i.e. binding to cellular CD4, to the major HIV-1 coreceptors CXCR4 or CCR5 for

X4 and R5 viruses [6], respectively, and fusion with cell membranes [7–15]. Results presented here show that CAP pretreated HIV-1 has a reduced capacity to bind to the coreceptors leading to impaired virus infectivity.

Methods

Reagents

The following monoclonal antibodies (mAbs; the source is indicated in parentheses) were used: 2F5 and 588D (Drs. T. Muster and S. Zola-Pazner, respectively); 9305 and 9284 (NEN Research Products, Du Pont, Boston, MA); b12, 2G12 and 17b (AIDS Research and Reference Reagent Program, Rockville, MD; courtesy of Drs. D. Burton, H. Katinger and J.E. Robinson, respectively) and anti-p24 (ImmunoDiagnostics, Inc., Woburn, MA). Rabbit antibodies against peptides from HIV-1 IIIB gp120/gp41 and against the V3 loop of HIV-1 BaL (anti-V3 BaL) were prepared as described [16]. Antiserum to phthalate was prepared by immunization of rabbits with phthalic anhydride treated rabbit serum albumin [17]. Recombinant soluble CD4 (sCD4) was from Genentech Inc., South San Francisco, CA. Recombinant HIV-1 IIIB and MN gp120, biotinylated gp120 and biotinylated sCD4 were from ImmunoDiagnostics Inc. Protein A, the protease inhibitors phenylmethyl-sulfonyl fluoride, leupeptin and pepstatin, and 2,3-bis [2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide (XTT) were all from Sigma, St. Louis, MO. Pelleted, 1000-fold concentrates of HIV-1 IIIB (6.8×10^{10} virus particles/ml) and BaL (1.8×10^{10} virus particles/ml) were from Advanced Biotechnologies Inc., Columbia, MD. Chicken serum was from OEM Concepts, Toms River, NJ. Horseradish peroxidase (HRP)- and phycoerythrin (PE)-labeled streptavidin were from Amersham, Arlington Heights, IL and R & D Systems, Minneapolis, MN, respectively. HRP was quantitated using a kit from Kirkegaard and Perry Laboratories Inc., Gaithersburg, MD. Enzyme linked immunoassays (ELISA) kits for the HIV-1 p24 antigen and for the β -gal protein were from Coulter Immunology, Hialeah, FL and 5 Prime \rightarrow 3 Prime Inc., Boulder, CO. CAP was a gift from Eastman, Kingsport, TN. H9 cells chronically infected with HIV-1 IIIB, HeLa-CD4-LTR- β -gal cells, GHOST CXCR4 and CCR5 cells and PM1 cells were obtained from the AIDS Research and Reference Reagent Program contributed by Drs. R. Gallo, M. Emerman, D. Littman, P. Lusso and M. Reitz, respectively. The Centricon centrifugal ultrafiltration devices were from Amicon/Millipore, Bedford, MA.

Measurement of HIV-1 infectivity

Serial two-fold dilutions of CAP treated and untreated HIV-1 IIIB (undiluted to 1/512) in RPMI-1640 medium containing 10% fetal bovine serum (FBS) were mixed with MT-2 cells (10^4 cells/well) and placed into 96-well polystyrene plates. The mixtures were incubated for 1 h

at 37°C and the volume was adjusted with RPMI-1640 medium containing 10% FBS to 200 μ l. On the 4th and 6th day after incubation at 37°C, 100 μ l of culture supernatants were removed from each well and equal volumes of fresh medium were added. On the 6th day, XTT dye (1 mg/ml) was added to the cells, Intracellular formazan was determined spectrophotometrically [18,19]. Similar experiments were done with HIV-1 BaL, except that PM1 cells were used instead of MT-2 cells, and virus production was measured by ELISA for p24 antigen one week after infection. The percentage of residual infectivity after CAP treatment was calculated from calibration curves relating absorbance (corresponding to formazan for HIV-1 IIIB and p24 antigen for HIV-1 BaL, respectively) to virus dilutions of untreated viruses. The results were plotted in Fig. 1.

Enzyme-linked immunosorbent assays (ELISA)

For virus capture assays, wells of 96-well polystyrene plates (Immulon II; Dynatech Laboratories Inc., Chantilly, VA) were coated either with sCD4 (1 μ g/well) or with monoclonal or polyclonal antibodies. For coating with antibodies, wells were first coated with protein A (1 μ g/well) in 0.1 M Tris buffer, pH 8.8 for 2 h at 20°C followed by either mAbs (1 μ g/well) or rabbit antisera diluted 1:100 in phosphate buffered saline (PBS) for 1 h at 20°C. Subsequently, the wells were washed and postcoated for 1 h at 20°C with bovine serum albumin (BSA) and gelatin (1 and 0.1 mg/ml in 0.14 M NaCl, 0.01 M Tris, pH 7.0 [TS]). Chicken serum (10%) in PBS (Ch-PBS) was used instead in experiments with HIV-1 BaL. The wells were washed with TS and stored at 4°C. HIV-1 virus particles suspended in PBS were added to the wells for 5 h at 4°C. Subsequently, the wells were washed 10 \times with ice cold PBS or 1:50 anti-p24 mAb in Ch-PBS for HIV-1 BaL, and then treated with lysis buffer (1% Nonidet P40 [NP40], 100 μ g/ml BSA in PBS) for 30 min at 37°C. The supernatants were removed and tested for p24 antigen using ELISA kits from Coulter Immunology and following the manufacturer's protocol. In other experiments, wells were coated with CAP (1 μ g/ml) in 25 mM sodium acetate, pH 6.0 and postcoated as described above.

To detect binding of CD4 to CAP treated and control gp120, wells coated with graded amounts of gp120 and postcoated as described above were reacted with biotinylated sCD4 (1 μ g/well) in PBS containing 100 μ g/ml BSA for 18 h at 4°C, washed with TS and the bound biotinylated CD4 was determined after adding HRP-streptavidin (1 μ g/ml) in TS containing 0.25% gelatine and 0.05% Tween 20 for 30 min at 37°C. The wells were washed and bound HRP was detected using the test kit from Kirkegaard and Perry and the absorbance read at 450 nm.

Flow cytometry

To determine the binding of gp120–biotinylated CD4 complexes to HIV-1 coreceptor expressing cells, CAP treated and untreated gp120 (5 µg) and biotinylated CD4 (2.5 µg) in PBS containing 100 µg/ml of BSA were mixed for 1 h at 20°C and then added to 10⁶ MT-2 cells in RPMI-1640 medium containing 100 µg/ml of BSA. Biotinylated CD4–gp120 complexes were not added to control cells. After 30 min at room temperature, the cells were washed 3 times with PBS containing 100 µg/ml of BSA, and PE-streptavidin (0.1 µg) was added. After 20 min at 20°C, cells were washed and fixed in 1% formaldehyde in PBS. Flow cytometry analysis was performed in a FACSCalibur flow cytometer (Becton Dickinson Immunocytometric Systems, San Jose, CA). Similar experiments were done with peripheral blood lymphocytes (PBL) isolated by the Isopaque-Ficoll technique [20].

Quantitation of CAP–gp120 binding

Forty µg of gp120 were mixed with 40 µg of CAP in 1 ml TS or 1 M NaCl, 0.01 M Tris, pH 7.0 and incubated at 20°C for 30 min. The mixtures were transferred into a Centricon centrifugal ultrafiltration device with a M_w cut-off of 100,000 and centrifuged at 3,500 × g for 30 min. The filtrates were transferred to a similar device with a M_w cut-off of 50,000 and centrifuged under the same conditions. CAP retained on top of the filter was quantitated as a complex with ruthenium red [21]. The retentate on the 100,000 M_w cut-off filter was dissolved in 1 ml of 2 M guanidinium hydrochloride and CAP released from the CAP–gp120 complex and retained on the 50,000 M_w cut-off filter was quantitated by the same method.

Molecular Modeling

A cellulose chain consisting of one cellotetraose unit (composed of four 1,4-linked β-D-glucose units) was created in Quanta [22] and 50% of the hydroxyl groups at positions 2- and 3- were modified to acetyl ester and 25% of the hydroxyl groups at position 6 were modified to phthaloyl ester [1,23]. The acetylated and phthaloylated cellotetraose structure (CTAP) was minimized by the steepest descent method followed by the adopted basis Newton–Raphson (ABNR) method. The energy change of 0.05 Kcal/mol between two successive structures was used as the termination criterion in both the steps.

The crystal structure of gp120 (1gcl) [13] was retrieved from the pdb ([http://www.rcsb.org]) and the V3 loop, created by homology modeling {(based on the nmr structure of the V3 loop from 1ce4) using the SWISS-MODEL [24] automated comparative protein modeling server ([http://www.expasy.ch/SWISS-MODEL.html]) } was attached to the gp120 crystal structure using Quanta's protein design module.

The docking simulation of CTAP onto the entire gp120 protein surface was performed by the Dockvision program [25]. A grid box (125 Å × 125 Å × 125 Å) with grid stepsize of 0.5 Å was created to cover the entire protein surface with enough area for the ligand to dock. The default forcefield (Research Potential Function) was used to perform 1000 Monte Carlo runs for the docking. Both ligand and the target protein were kept rigid. Intermolecular energy criteria were used to identify the best possible dockings of CTAP.

Results

Impaired infectivity of CAP treated HIV-1

Results of earlier studies indicated that HIV-1 infection of cells is inhibited in the presence of CAP (ED₉₀ = 5 to 10 µg/ml for HIV-1 IIIB, i.e. < 200 nM) [2]. However, the mechanism involved in the inhibitory activity and the possibility of virus inactivation by CAP have not been explored. Results shown in Fig. 1 indicate that CAP in a dose dependent manner rapidly inactivates at 37°C HIV-1 IIIB, an X4 virus and HIV-1 BaL, an R5 virus, the latter appearing relatively more resistant.

CAP retention on the surface of treated HIV-1 particles

Evidence for CAP–HIV-1 binding was obtained from results of solid phase immunoassays in which attachment of HIV-1 IIIB and BaL virus particles, respectively (detected by subsequent ELISA of p24 antigen released from virus particles by detergent treatment) to wells pre-coated with CAP was measured (Fig. 2A). CAP treated HIV-1 IIIB and BaL viruses, unlike control viruses, bound to wells coated with antibodies against phthalate (Fig. 2B). These results indicate that treated HIV-1 particles, utilizing either CXCR4 or CCR5 as coreceptors, retain CAP on their surface and suggest that this is responsible for the altered properties and impaired infectivity of the treated viruses.

Identification of CAP binding sites on HIV-1 envelope glycoproteins

The attachment sites on the surface of HIV-1 IIIB for CAP were determined from binding of control and CAP treated HIV-1, respectively, to distinct ligands (Fig. 3). The quantities of control virus and treated virus in these experiments were identical, as determined from the content of p24 antigen in preparations of detergent-disrupted virus particles. CAP binding to HIV-1 IIIB particles most profoundly affected their binding to the V3 loop specific mAbs 9284 [26] and 9305 [27] and to the coreceptor CXCR4, while binding to sCD4; to virus neutralizing mAbs specific for the CD4 binding site on gp120, b12 [28] and 588D [29]; to mAb 17b specific for a discontinuous conserved epitope proximal to the binding site for both CD4 and anti-CD4 binding site antibodies [30]; to mAb 2G12 specific for an epitope centered around the

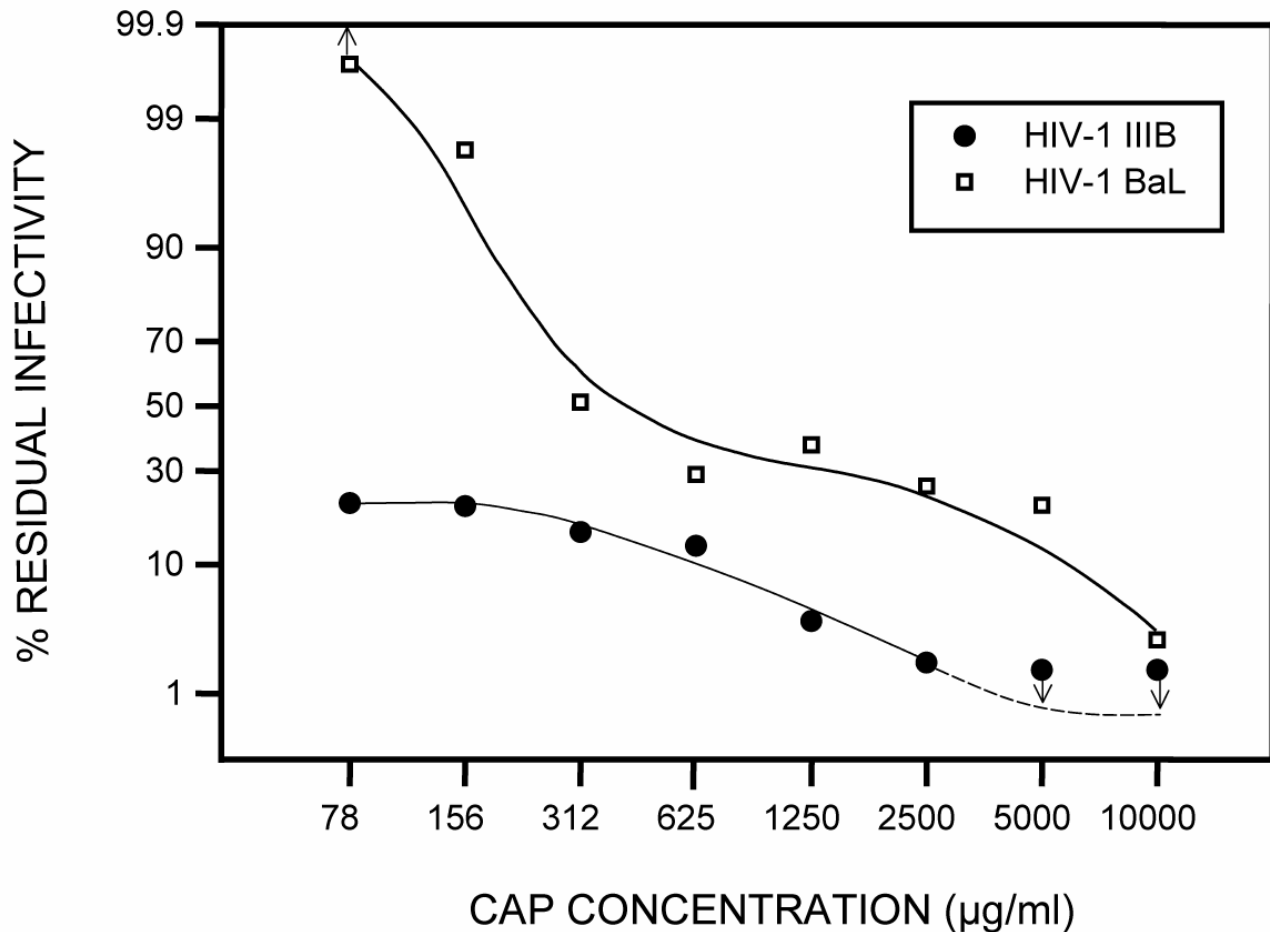


Figure 1

Inactivation of HIV-1 by CAP. Cellulose acetate phthalate (CAP) (final concentrations between 10 and 0.078 mg/ml) was added to HIV-1 IIB containing tissue culture medium and to HIV-1 BaL, respectively. After incubation for 5 min at 37°C, the mixtures were cooled on ice and a solution of polyethylene glycol 6000 (PEG) [Reference 53] was added to a final concentration of 3% to separate HIV-1 from CAP (which does not precipitate in 3% PEG). After 90 min at 4°C, the mixtures were centrifuged at 10,000 rpm, the supernatant fluids removed and the pellets washed twice with 3% PEG in PBS containing 10 mg/ml BSA. The final pellets were resuspended in tissue culture medium and titered for infectivity. The percentage of residual infectivity is shown in a probability scale.

C3/V4 domain of gp120 also involving N-linked glycans [31] and to the gp41 specific mAb 2F5 [32] was less affected. In order to determine whether R5 viruses are similarly affected by CAP, the binding of HIV-1 BaL as a representative of this virus group to anti-V3 BaL and CCR5, respectively, before and after CAP treatment was studied. The results shown in Fig. 3 indicate that the CAP-treated HIV-1 BaL bound to these ligands much less than did untreated virus.

The binding of control and CAP-treated HIV-1 IIB to antibodies against peptides derived from gp120/gp41 [16] was also determined. In agreement with the results ob-

tained using mAbs, CAP treatment resulted in most pronounced decreases of virus binding to antibodies against peptide 303–338 (= V3 loop) and the adjacent peptide 280–306 (Fig. 4).

Pretreatment with CAP impairs gp120 binding to coreceptors

Data reported so far suggest that the lethal hit to HIV-1 caused by CAP treatment involves the coreceptor binding site on gp120. To further support this conclusion, the binding of labeled gp120–CD4 complexes to coreceptor [11,12,33] expressing cells was studied. First, it was determined from quantitative binding studies using CAP

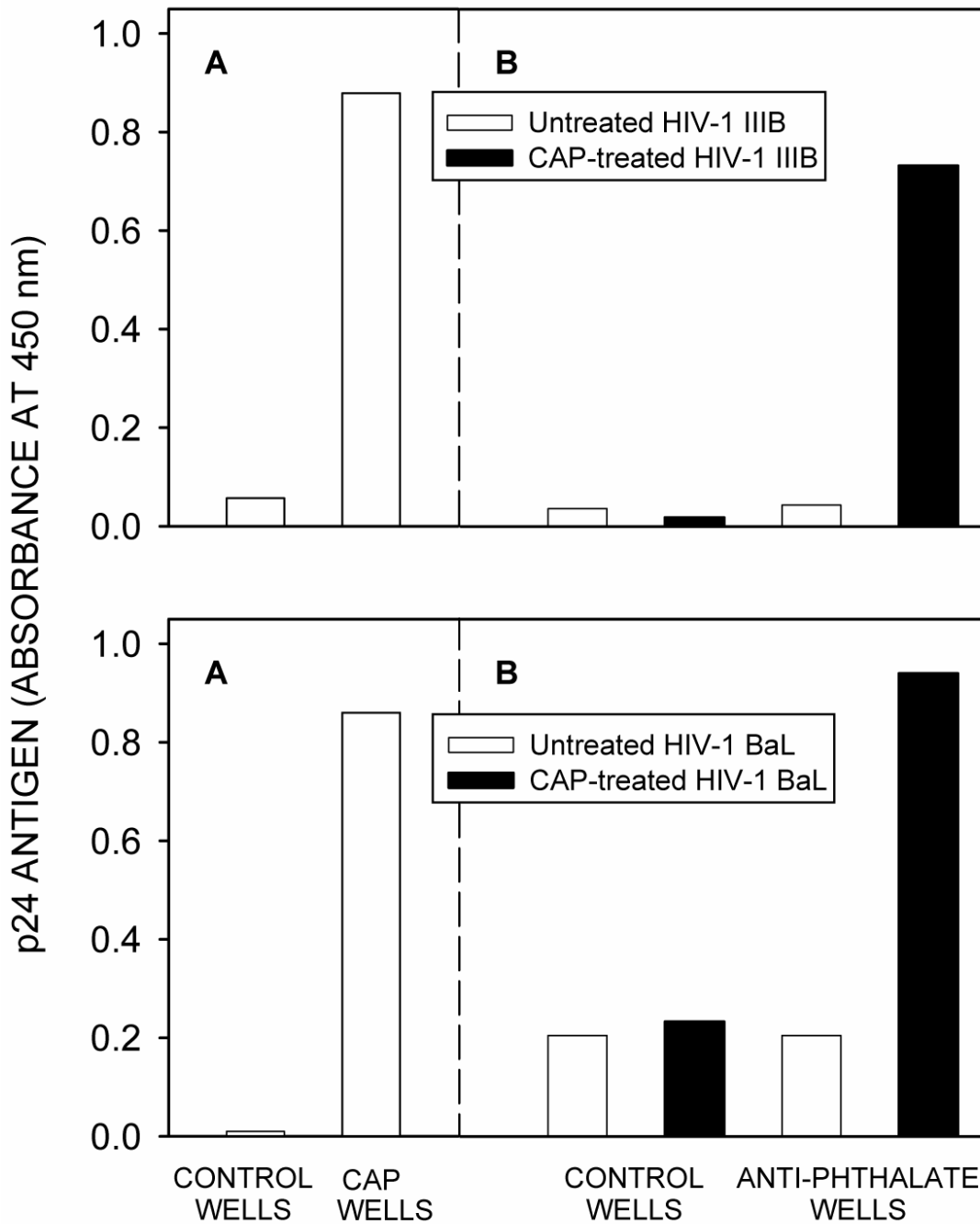


Figure 2

Evidence for CAP binding to HIV-1 virus particles. A) Virus binding to CAP coated wells. B) Binding of CAP treated and untreated HIV-1 IIIIB and BaL, respectively, to wells coated with antibodies against phthalate [reference 17]. CAP was added to a final concentration of 5 mg/ml to 50 μ l of suspensions of purified HIV-1 IIIIB (6.8×10^9 virus particles per ml) and HIV-1 BaL (1.8×10^{10} virus particles per ml), respectively, in 0.1 M sodium acetate pH 7.0. CAP was not added to control virus preparations. After 5 min at 37°C, HIV-1 was separated from unbound CAP as described for Fig. 1. The pellets containing HIV-1 were resuspended in 50 μ l PBS containing 100 μ g/ml BSA. 200 μ l of 5-fold diluted virus suspensions containing equal amounts of virus particles for both CAP treated and control virus (as determined by quantitation of p24 antigen), were added to wells coated as indicated above and to control wells. Bound virus was quantitated by ELISA for p24 antigen.

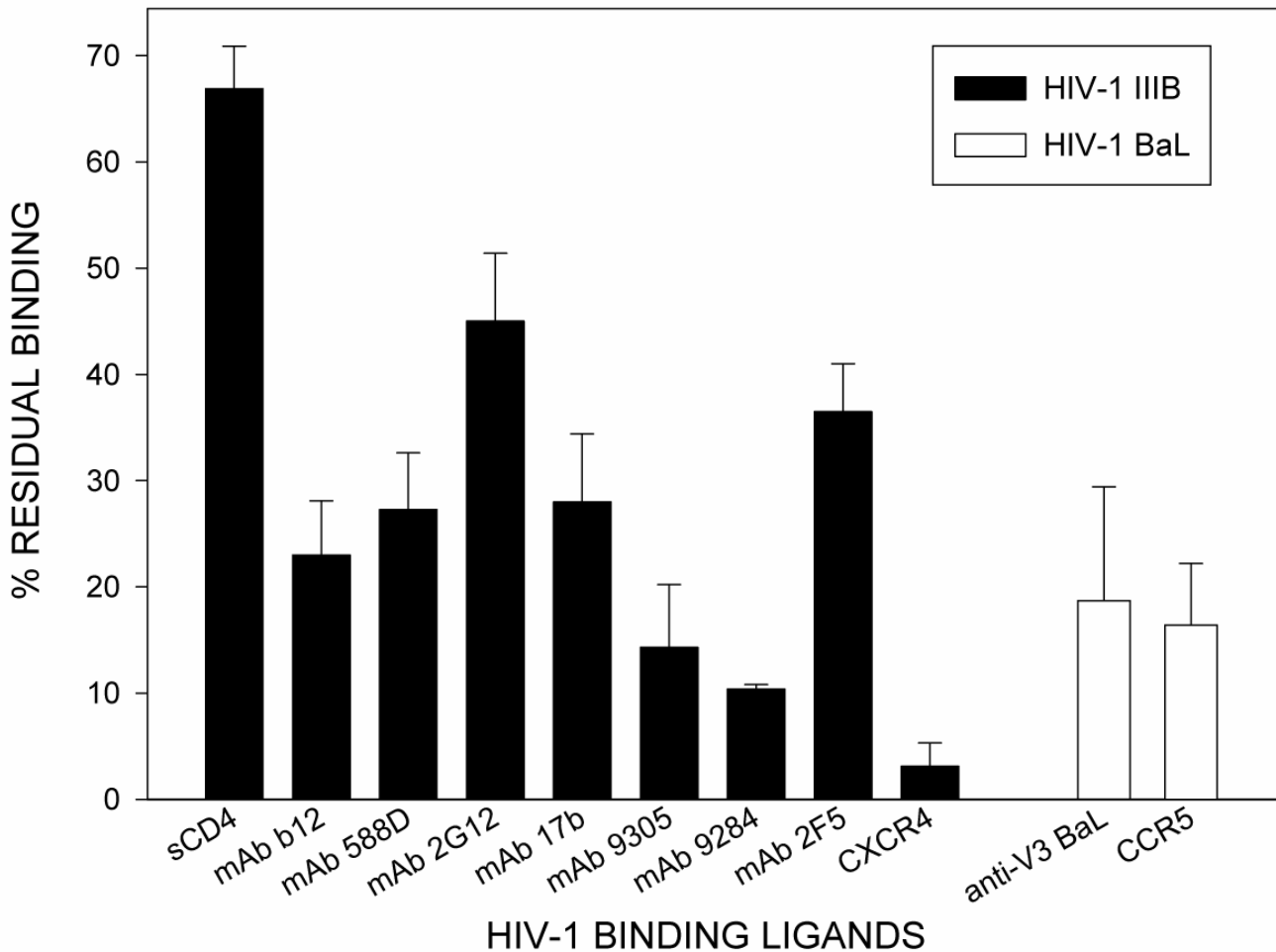


Figure 3

Binding of CAP treated and untreated HIV-1 to distinct ligands. The binding of untreated and CAP treated HIV-1 IIIIB to wells coated with sCD4 or with distinct mAbs and of HIV-1 BaL to anti-V3 BaL was measured as described for Fig. 2. The % of binding corresponding to CAP treated virus was calculated based on the formula (% residual binding = [absorbance corresponding to bound CAP treated virus ÷ absorbance corresponding to bound untreated HIV-1] × 100. Absorbance corresponding to p24 antigen (5-fold dilution of the sample) from untreated HIV-1 bound to the respective ligands was in the range of 0.56 to 1.54. Absorbance corresponding to virus captured onto wells coated with control IgG was 0.046. All experiments were done in triplicate. To measure virus binding to the coreceptor CXCR4, treated and control HIV-1 IIIIB recovered after PEG precipitation was mixed with 10 µg of sCD4. After 5 min at 20°C, the respective samples were divided into 2 aliquots, each of which was added to 5 × 10⁵ GHOST CXCR4 cells suspended in 100 µl PBS containing 100 µg/ml of BSA. Similar experiments were carried out with purified HIV-1 BaL, except that GHOST CCR5 cells were used. After 1 h at 4°C, the cells were pelleted and washed with ice cold PBS containing 100 µg/ml BSA. The pelleted cells were lysed for 30 min at 37°C in PBS with 1% NP40. Serial 5-fold dilutions in PBS (1:5 to 1:4.9 × 10⁷) were tested by ELISA for the p24 antigen. Changes in HIV-1 binding were determined using calibration curves relating absorbance to virus dilutions.

staining with ruthenium red [21] that pretreatment with CAP resulted in binding of 0.90 ± 0.13 CAP molecules/gp120 in TS. The binding appeared augmented by ~ 30% in 1 M NaCl. The binding capacity of gp120 for sCD4 was preserved after CAP treatment (Fig. 5). On the other hand, gp120–sCD4 complexes containing CAP treated gp120 bound to coreceptor expressing cells to a much

lesser extent than similar complexes containing untreated gp120 (Fig. 6).

Discussion

Negatively charged sulfated polymers were reported to have anti-HIV-1 activity and are being considered for development as topical microbicides. They include: dex-

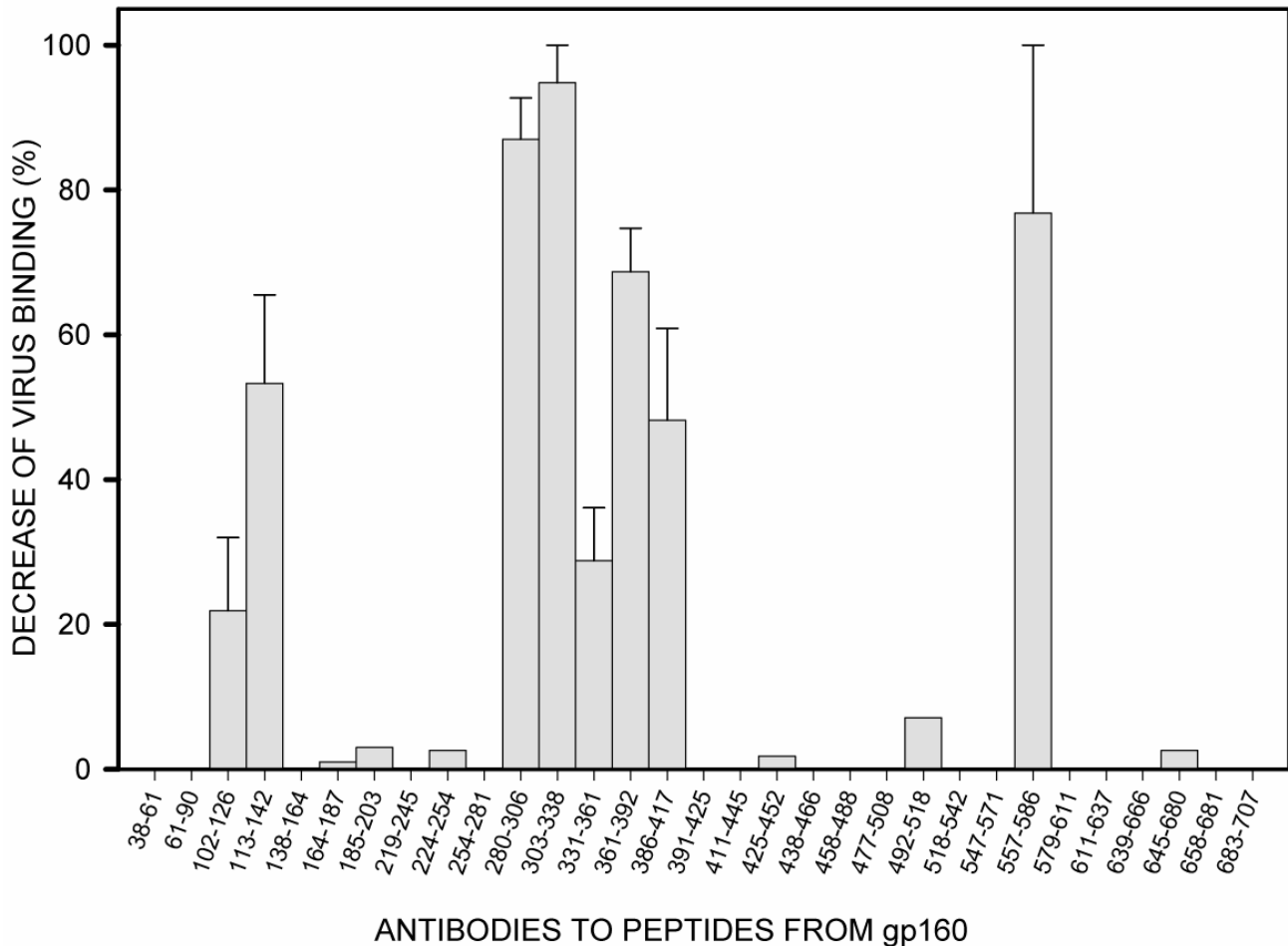


Figure 4

Binding of CAP treated and untreated HIV-1 IIB to wells coated with antibodies to peptides from gp120/gp41 [reference 16]. Experimental conditions were similar to those described in the legend for Fig. 3. The absorbance corresponding to untreated HIV-1 captured onto the wells was in the range of 0.09 to 0.37. The absorbance corresponding to controls (virus captured onto wells coated with Protein A followed by normal rabbit serum) was 0.014. Numbering of gp160 amino acid residues was the same as in reference 16. Decreases of CAP treated virus binding, as compared with binding of control virus, were plotted.

tran sulfate [34,35], carrageenans [35,36], dextrin-2-sulfate [37,38], cellulose sulfate [39] and naphthalene sulfonate polymer (PRO 2000; [40]). Except for dextrin-2-sulfate and PRO 2000, which appear to have anti-HIV-1 inhibitory activities similar to that of CAP, the other sulfonated polymers are less inhibitory [34,35,41]. HIV-1 may develop resistance to the inhibitory effect of dextran sulfate and R5 viruses were reported not to be inhibited by this polymer [42,43]. Dextran sulfate was shown to bind to gp120 and to interfere with gp120/CXCR4 interactions but it did not bind to gp120 of R5 viruses [43].

CAP in micronized form, providing an acidic environment, causes disintegration of HIV-1 leading to loss of infectivity (2). Here we have shown that CAP directly in-

activates HIV-1 at neutral pH since treated virus particles after removal of excess CAP had reduced or no infectivity (Fig. 1), in agreement with recent observations [44,45]. Virus inactivation can be attributed to strong binding of CAP to HIV-1, preventing the access to virus particles predominantly of antibodies against the gp120 V3 loop, known to be involved in coreceptor binding and specificity [15,46–49], and to epitopes located adjacent to the V3 loop (Fig. 4). Indeed, CAP binding to HIV-1 resulted in blockade of sites involved in coreceptor binding (Fig. 3). This was observed for both HIV-1 IIB, an X4 virus, and HIV-1 BaL, an R5 virus. In agreement with this finding, CAP inhibited infection by both X4 and R5 viruses in *in vitro* models for vaginal HIV-1 infection [45,50]. Sites on the HIV-1 envelope other than the core-

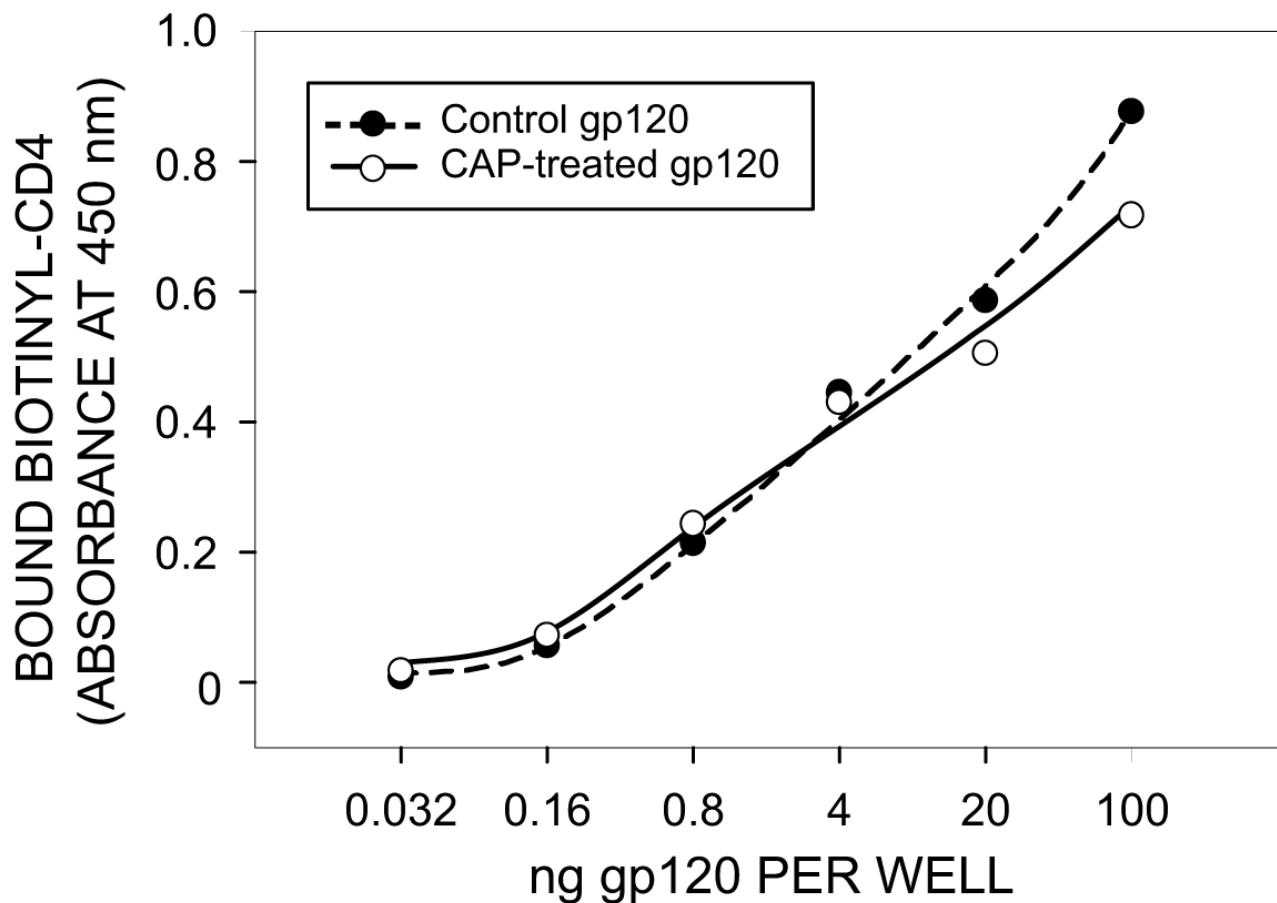


Figure 5

Binding of sCD4 to CAP treated and untreated gp120. Recombinant gp120 IIIB (5 μ g in 400 μ l of 0.1 M sodium acetate buffer pH 7.0) was treated with CAP (5 mg/ml) for 5 min at 37°C and then cooled to 0°C. CAP was omitted in control experiments. BSA was added to a final concentration of 25 μ g/ml and the samples were filtered using a 2 ml Centricon centrifugal ultrafiltration device with a M_w cutoff of 100,000 and centrifuged at 3,500 \times g for 30 min. gp120 retained on the filters was washed with PBS, resuspended in PBS, and serially diluted 5-fold. The diluted samples (corresponding to gp120 quantities indicated on the abscissa) were used to coat wells of 96-well polystyrene plates. Binding to the wells of biotin labeled sCD4 was detected from subsequent binding of HRP-streptavidin.

ceptor binding domains may possibly be also involved in interactions with CAP, contributing to inhibition of virus infection.

Studies with gp120 allowed to determine the stoichiometry of the reaction between gp120 and CAP, resulting in the observation that approximately one CAP molecule binds to one molecule of gp120. Interestingly, CAP-gp120 binding also occurs in 1M NaCl, conditions under which binding of sulfated polysaccharides to gp120 is abolished [43]. This suggests that electrostatic interactions do not play an exclusive role in CAP-gp120 binding [51].

Since CAP has an approximate M_w of 6×10^4 and its structure has not been determined, a model for CAP docking onto gp120 (HXBc2) was generated using a chemically modified cellotetraose. The entire gp120 surface was considered for docking acetylated and phthaloylated cellotetraose (CTAP) except the trimerization and CD4 binding sites on gp120 [13]. The two optimally docked CTAP molecules were selected for further analyses. The first one (marked as *Dock1* in Fig. 7) docked near regions corresponding to amino acid residues 361–392 (indicated in red) and 386–417 (indicated in purple). The orientation of CTAP revealed that if extended to generate the larger CAP molecule, blockade of the coreceptor binding site (indicated in gray in Fig. 7)

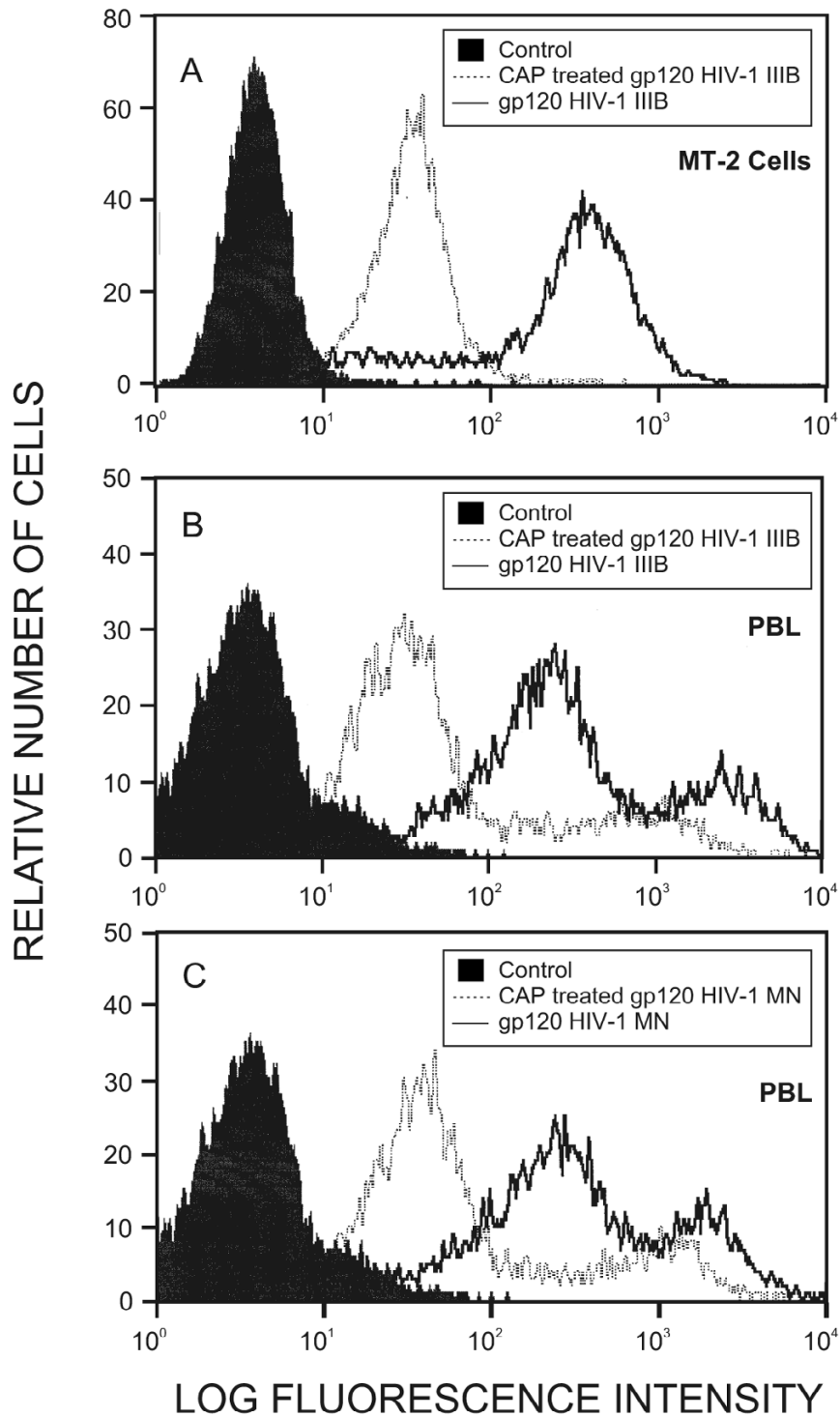


Figure 6

Binding of sCD4 complexes with CAP treated and control gp120, respectively, to HIV-1 coreceptor expressing cells. CAP treated or control gp120 IIIB or MN (prepared as described for Fig. 5) were mixed with biotinylated sCD4 and added to 10⁶ MT-2 cells (A) or PBL (B and C). The cells were washed with PBS, treated with phycoerythrin (PE)-labeled streptavidin, fixed in 1% formaldehyde and submitted to flow cytometry analysis.

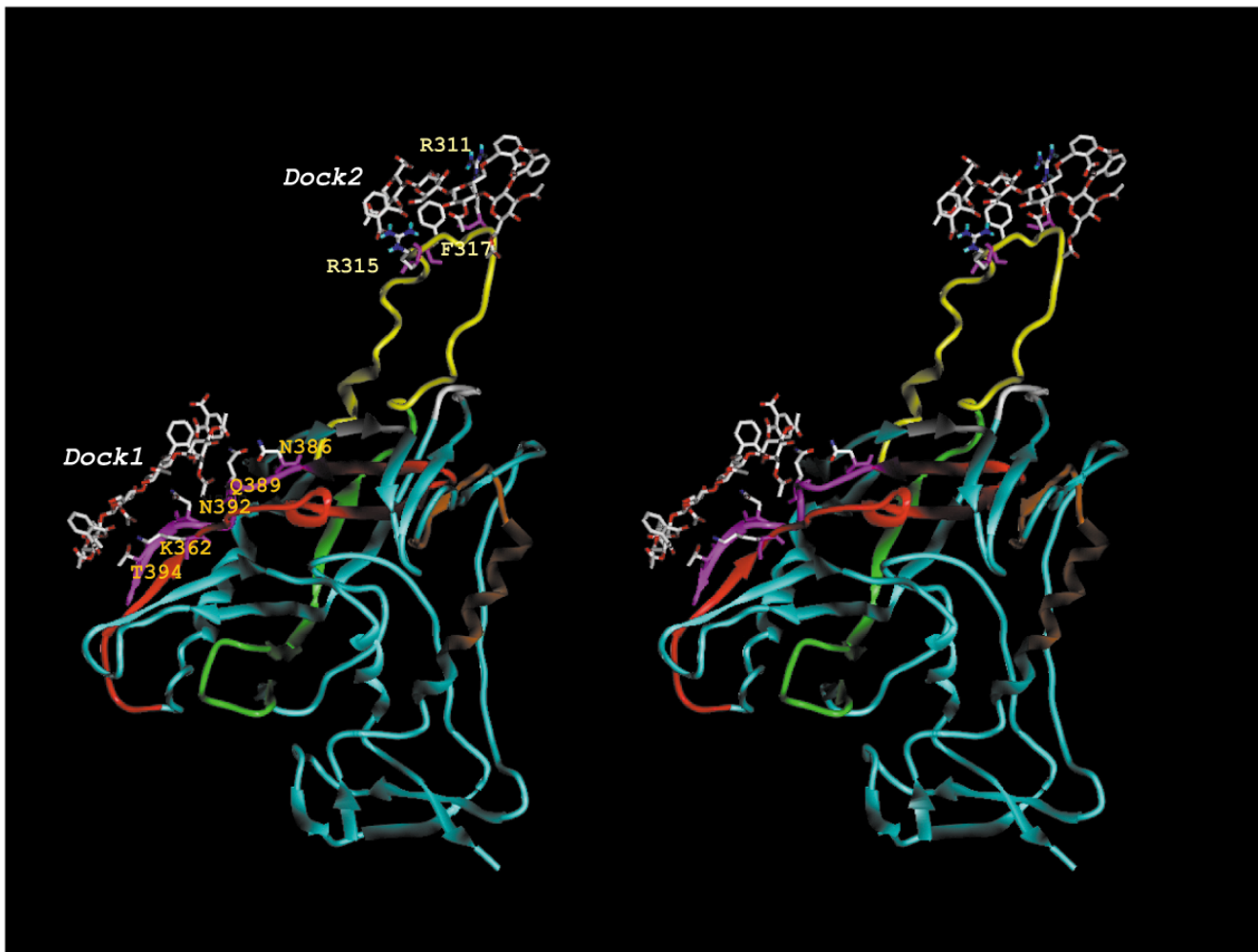


Figure 7

Stereodiagram of two best docked modified cellotetraose units (CTAP) (marked as *Dock1* and *Dock2*, respectively) on the x-ray crystal structure of gp120 (HXBc2 strain) with the V3 loop attached. The residues on gp120 nearest to the docked CTAPs are shown to indicate possible interaction patterns. The V3 loop (peptide 303–338) is indicated in yellow. Regions corresponding to peptides 113–142, 280–306, 361–392 and 393–417 (Fig. 4) are indicated in orange, green, red and purple, respectively. The coreceptor binding site is indicated in gray. The rest of the gp120 is in cyan. The figure was generated using the Sybyl program [54].

[15] and the V3 loop (indicated in yellow in Fig. 7) would result. These docking results agree with the observation that capture of CAP treated HIV-1 virions to antibodies against peptides from these regions (303–338, 361–392, 386–417) was decreased (Fig. 4). CTAP interacts primarily through several hydrogen bonds with gp120 residues K362, N386, Q389, N392 and T394. Though there were no hydrophobic or electrostatic interactions discernable, such interactions cannot be ruled out as CTAP represents only a small part of the CAP molecule. The second best docking positioned CTAP near the V3 loop (marked as *Dock2* in Fig. 7). CTAP in this position interacts with gp120 through both hydrogen bonding (residues R311

and R315) and hydrophobic forces (F317) with two CH_3CO^- units of CTAP. The interacting region is in the RGPGRF principal neutralizing domain (PND) of the V3 loop [52]. Both R311 and R315 residues are also in close proximity to two acid groups of the CTAP phthalic acid moiety and may take part in electrostatic interactions. Although CTAP is only a partial representation of the CAP molecule, the docking simulations agree with the experimental results.

Results presented earlier [2] combined with those presented here suggest that CAP acts on HIV-1 by several mechanisms, depending on pH and the physical form

and concentration of this compound. The direct virus inactivating effect of CAP, the multiple mechanisms involved in prevention of HIV-1 infection, the broad spectrum activity against several STD pathogens, the established safety record, low cost and availability in large scale suggest that CAP has advantages over other polymeric substances considered as topical (vaginal) microbicides for prevention of STDs, including AIDS.

Conclusions

Cellulose acetate phthalate (CAP) is a pharmaceutical excipient which has been used for over four decades for enteric film coating of tablets and capsules. It is inexpensive and generally regarded as a nontoxic material free of adverse effects. As such, it is included in the US Food and Drug Administration Inactive Ingredients Guide. Our earlier studies indicated that CAP inhibited infection by HIV-1 and several herpes viruses in vitro. CAP was effective in blocking vaginal transmission of these viruses in animal model systems. The underlying molecular mechanisms for these inhibitory effects remained to be established. Data presented here show that CAP remains bound to HIV-1 after removal of unreacted compound, impairing virus infectivity due to blockade of binding sites for cellular coreceptors CXCR4 and CCR5. The industrial availability, history of safe use and mechanism of anti-HIV-1 activity (i.e. virus inactivation rather than reversible inhibition) suggest that CAP is an ideal candidate compound for a vaginal microbicide expected to prevent sexual transmission of HIV-1.

List of Abbreviations used

CAP, cellulose acetate phthalate; STD, sexually transmitted disease; ELISA, enzyme-linked immunosorbent assay; mAbs, monoclonal antibodies; FBS, fetal bovine serum; PEG 6000, polyethylene glycol 6000; HIV-1, human immunodeficiency virus type 1; SIV, simian immunodeficiency virus; HSV-1, herpesvirus type 1; HSV-2, herpesvirus type 2; BSA, bovine serum albumin; PBS, phosphate buffered saline; HRP, horse radish peroxidase; sCD4, soluble CD4; TS, 0.14 M NaCl, 0.01 M Tris, pH 7.0; PBL, peripheral blood lymphocytes; CTAP, celotetraose acetate phthalate; pdb, Protein Data Bank.

Declaration of Competing Interests

None declared

Acknowledgments

We thank Ms. J. Pack and V. Kuhlemann for preparation of the manuscript and figures, Dr. S. Jiang for reading the manuscript and comments, and Ms. R. Croson-Lowney and Ms. S. Guerrero for flow cytometry. This study was supported by intramural institutional funds and the Marilyn M. Simpson Charitable Trust. A. K. Debnath had support from the Hugoton Foundation, Philip Morris Companies, Inc., and Johnson & Johnson, Inc.

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