BMC Infectious Diseases

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

BMC Infectious Diseases 2001, **1**:17

Received: 3 July 2001
Accepted: 25 September 2001

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

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 inactivating 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 ~ 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. Pelletted, 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 → 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 µl. On the 4^{th} and 6^{th} day after incubation at 37°C, 100 µl of culture supernatants were removed from each well and equal volumes of fresh medium were added. On the 6^{th} 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 µg/well) or with monoclonal or polyclonal antibodies. For coating with antibodies, wells were first coated with protein A (1 µg/well) in 0.1 M Tris buffer, pH 8.8 for 2 h at 20°C followed by either mAbs (1 µg/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 NaCI, 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 × 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 µg/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 µg/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 µg/well) in PBS containing 100 µg/ml BSA for 18 h at 4°C, washed with TS and the bound biotinylated CD4 was determined after adding HRP-streptavidin (1 µg/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 10^6 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 NaCI, 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 (ED_{90} = 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 precoated 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

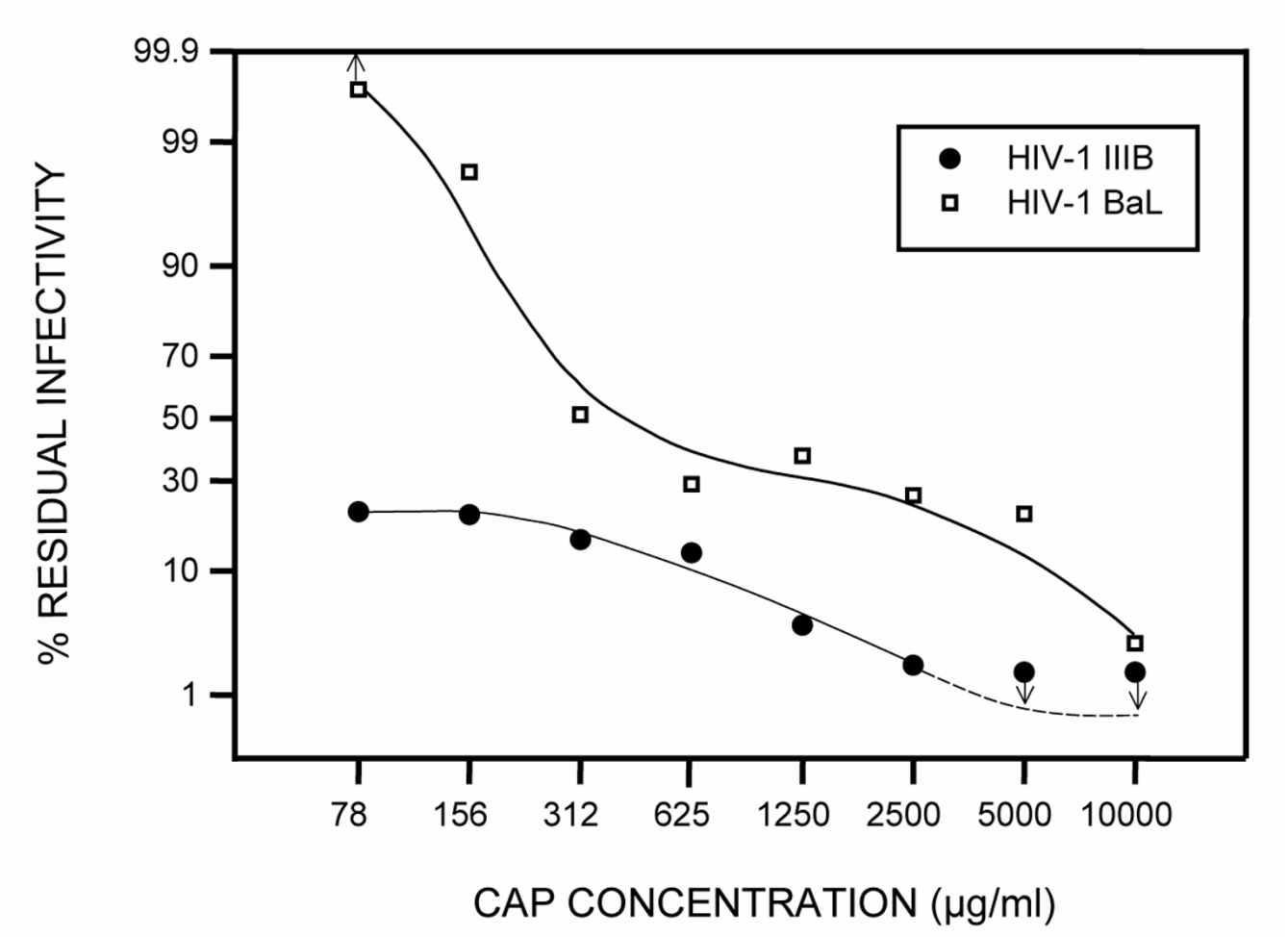

Figure 1
Inactivation of HIV-1 by CAP. Cellulose acetate phthalate (CAP) (final concentrations between 10 and 0.078 mg/ml) was added to HIV-1 IIIB containing tissue culture medium and to HIV-1 BaL, respectively. After incubation for 5 min at 37°C, the mixtures were cooled on ice and a solution of polyethylene 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 IIIB to antibodies against peptides derived from gp120/gp41 [16] was also determined. In agreement with the results obtained 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

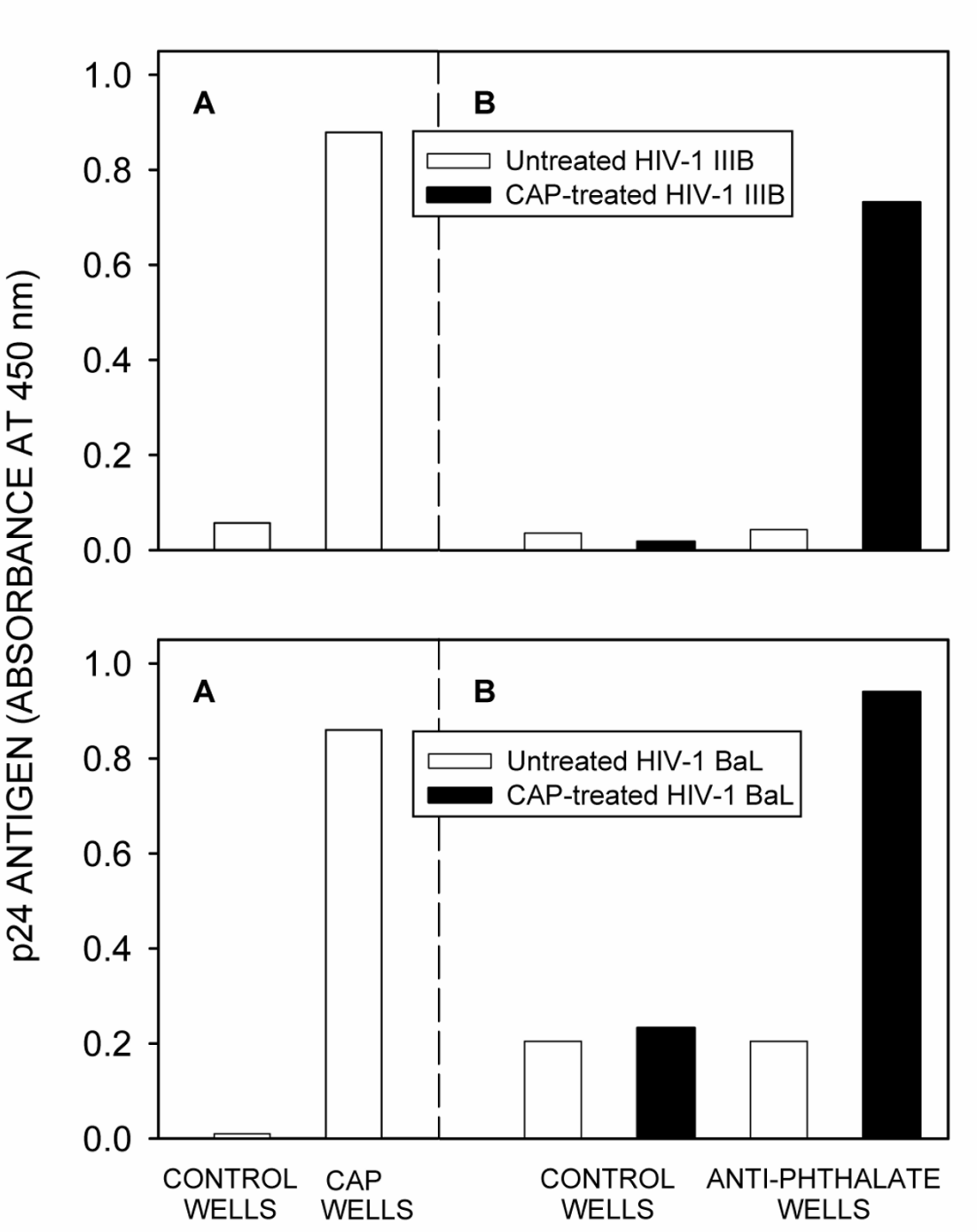

Figure 2
Evidence for CAP binding to HIV-1 virus particles. A) Virus binding to CAP coated wells. B) Binding of CAP treated and untreated HIV-1 IIIB and BaL, respectively, to wells coated with antibodies against phthalate [reference 17]. CAP was added to a final concentration of 5 mg/ml to 50 μl of suspensions of purified HIV-1 IIIB (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 μl PBS containing 100 μg/ml BSA. 200 μl of 5-fold diluted virus supensions 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.

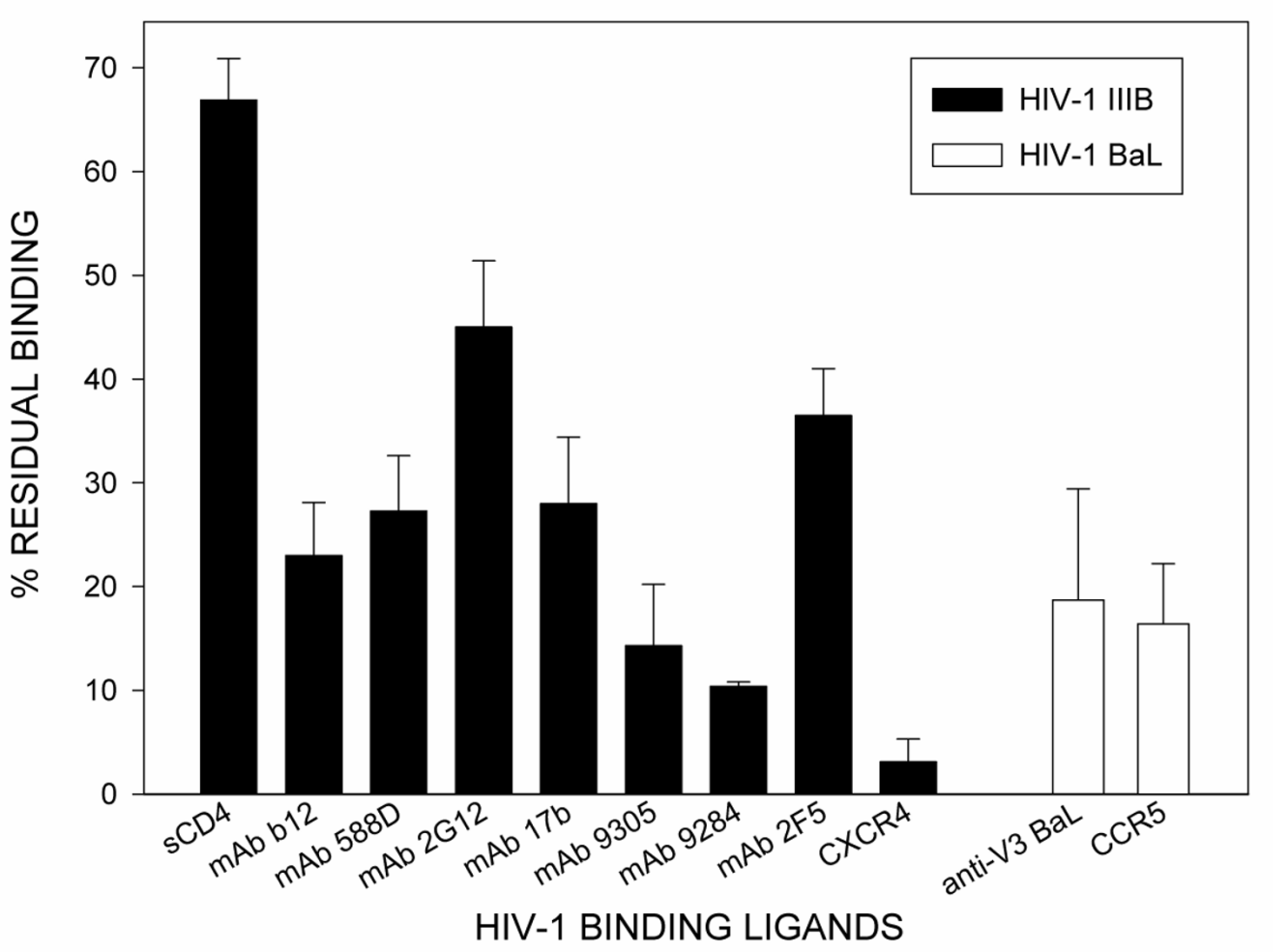

Figure 3
Binding of CAP treated and untreated HIV-1 to distinct ligands. The binding of untreated and CAP treated HIV-1 IIIB to wells coated with sCD4 or with distinct mAbs and of HIV-1 BaL to anti-V3 BaL was measured as described for Fig. 2. The % of binding corresponding to CAP treated virus was calculated 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 IIIB 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 5×10^5 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 pelletted and washed with ice cold PBS containing 100 μg/ml BSA. The pelletted cells were lysed for 30 min at 37°C in PBS with 1% NP40. Serial 5-fold dilutions in PBS (1:5 to $1:4.9 \times 10^7$) 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-

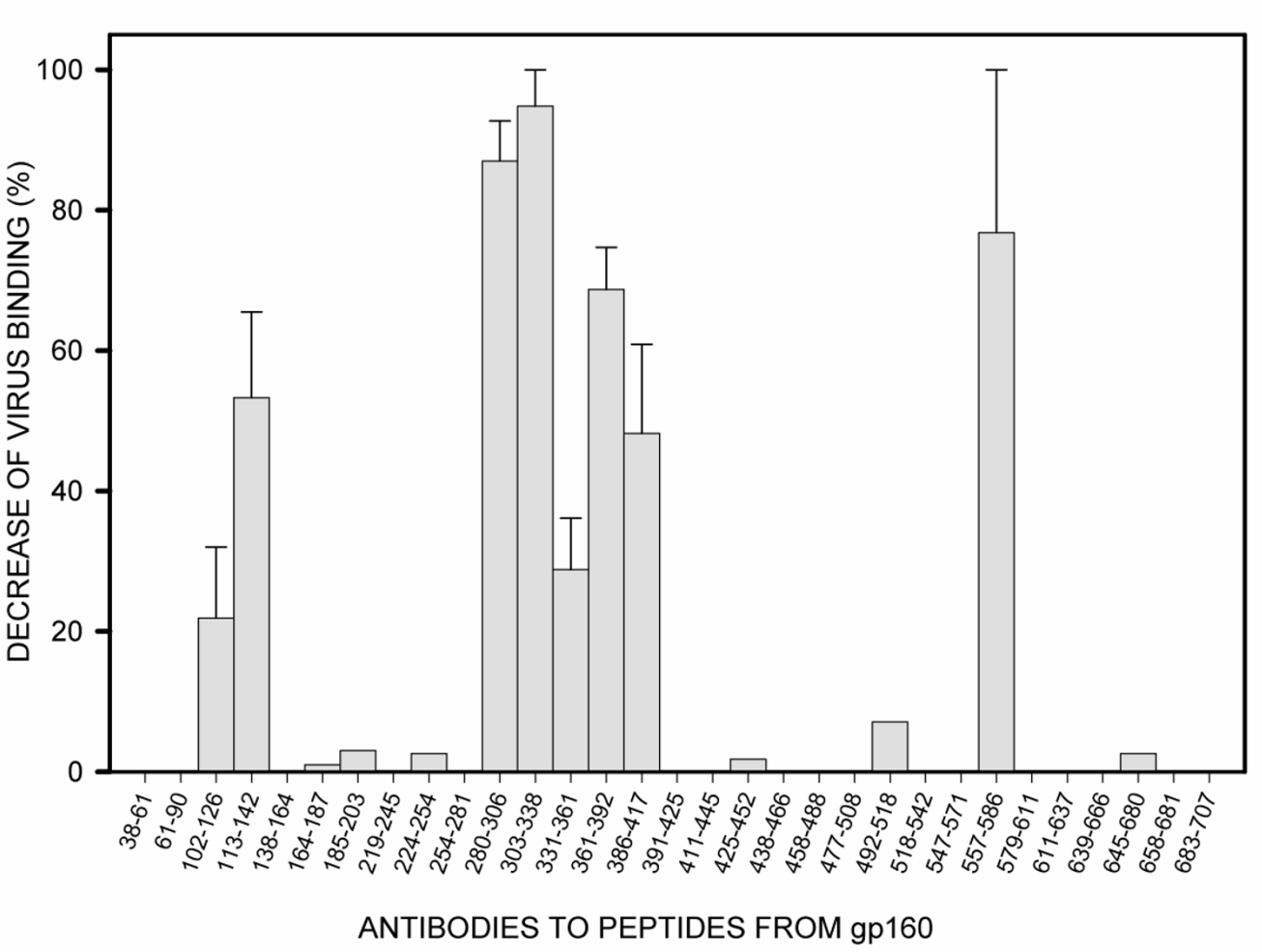

Figure 4
Binding of CAP treated and untreated HIV-1 IIIB to wells coated with antibodies to peptides from gp120/gp41 [reference 16]. Experimental conditions were similar to those described in the legend for Fig. 3. The absorbance corresponding to untreated HIV-1 captured onto the wells was in the range 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 inactivates 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 IIIB, 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-

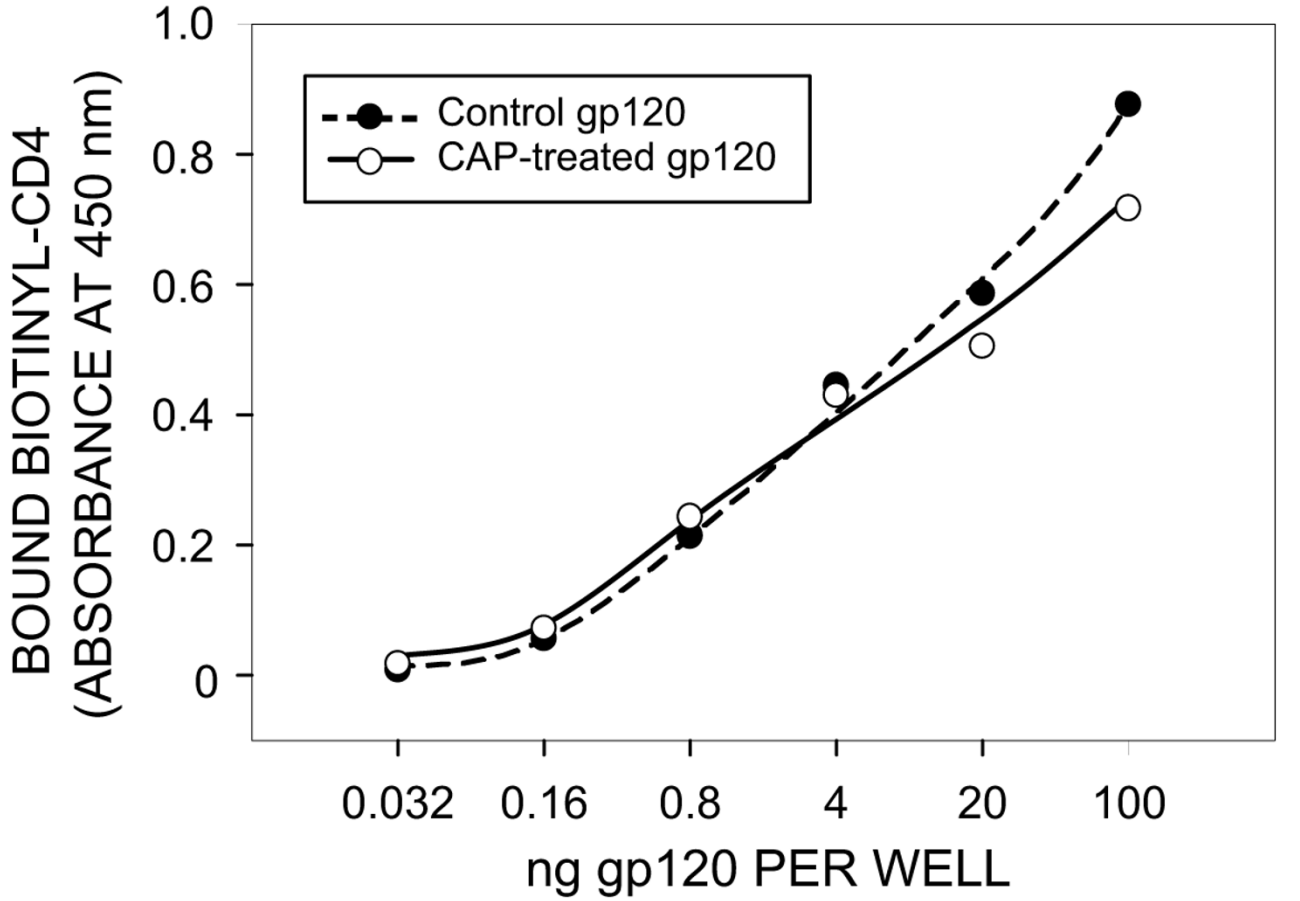

Figure 5
Binding of sCD4 to CAP treated and untreated gp120. Recombinant gp120 IIIB (5 μg in 400 μl of 0.1 M sodium acetate buffer pH 7.0) was treated with CAP (5 mg/ml) for 5 min at 37°C and then cooled to 0°C. CAP was omitted in control experiments. BSA was added to a final concentration of 25 μg/ml 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 × 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)

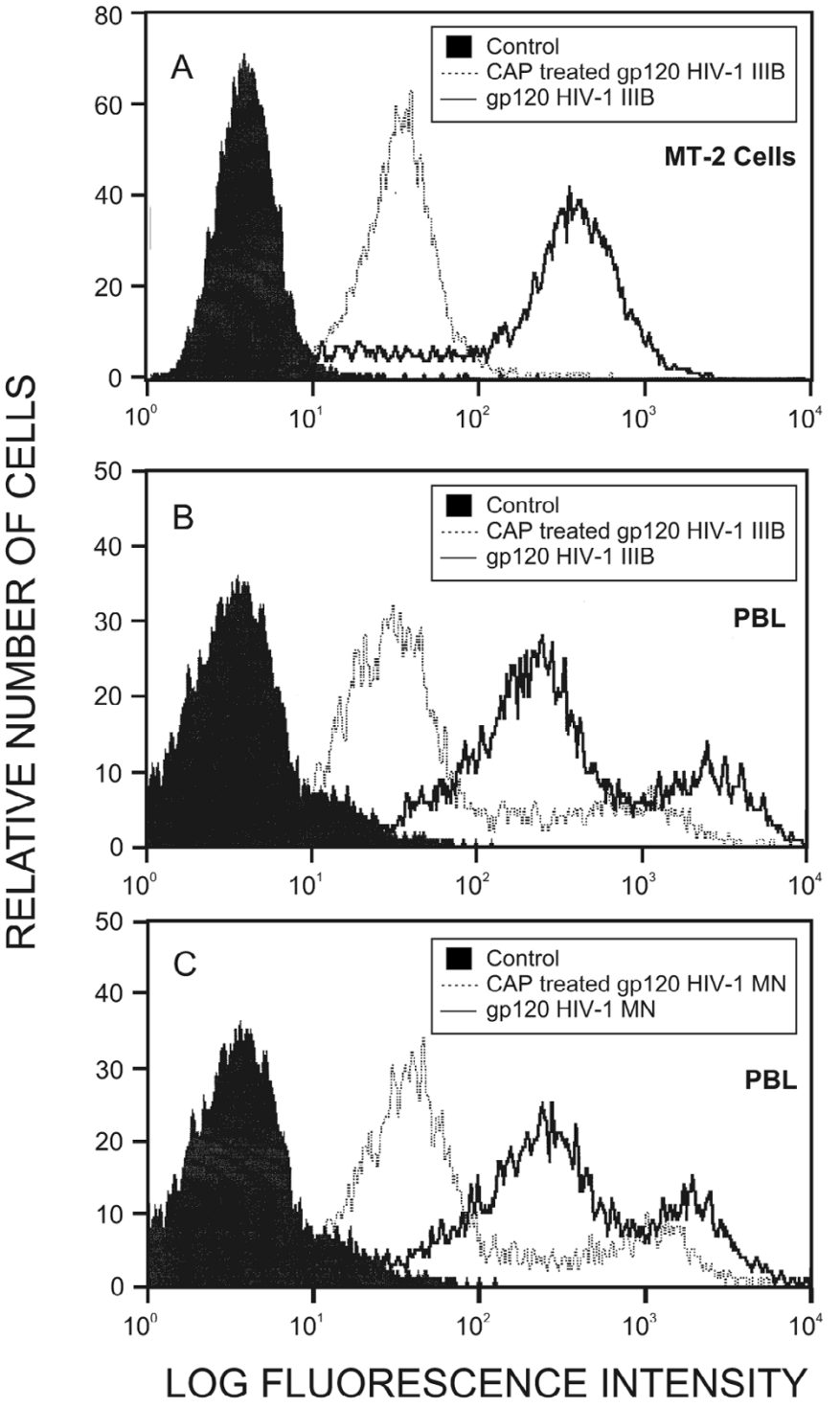

Figure 6
Binding of sCD4 complexes with CAP treated and control gp120, respectively, to HIV-1 coreceptor expressing cells. CAP treated or control gp120 IIIB or MN (prepared as described for Fig. 5) were mixed with biotinylated sCD4 and added to 10^6 MT-2 cells (A) or PBL (B and C). The cells were washed with PBS, treated with phycoerythrin (PE)-labeled streptavidin, fixed in 1% formaldehyde and submitted to flow cytometry analysis.

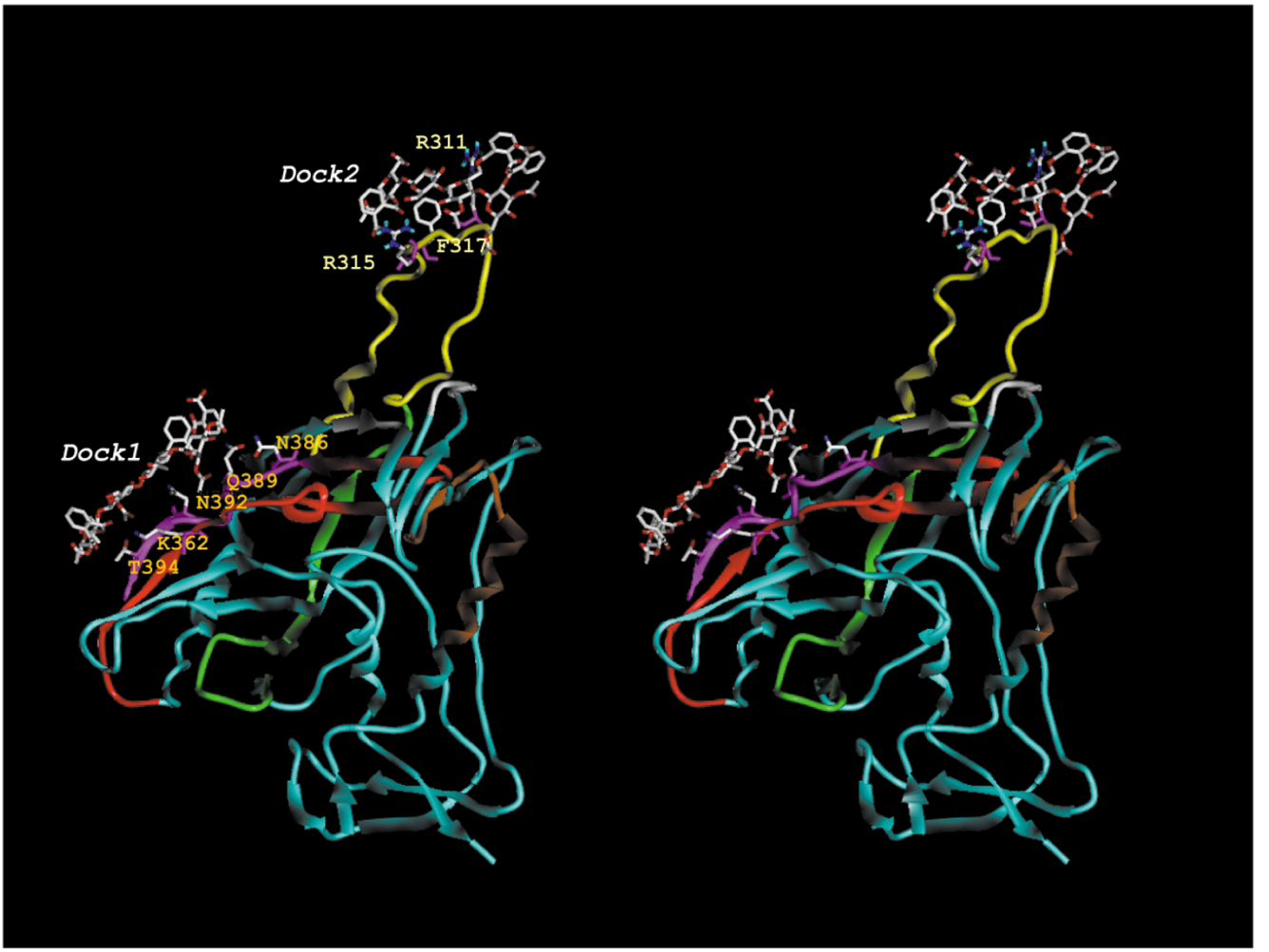

Figure 7
Stereodiagram of two best docked modified cellotetraose units (CTAP) (marked as *Dock1* and *Dock2*, respectively) on the x-ray crystal structure of gp120 (HXBc2 strain) with the V3 loop attached. The residues on gp120 nearest to the docked CTAPs are shown to indicate possible interaction patterns. 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 RGPGRAF 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 machanisms 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, cellotetraose 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.

References

1. Lee JC: **Cellulose acetate phthalate.** *In Handbook of Pharmaceutical Excipients. Edited by Wade A, Weller PJ. Washington, D.C.: American Pharmaceutical Association Pub.;* 199491-93
2. Neurath AR, Strick N, Li Y-Y, Lin K, Jiang S: **Design of a "microbicide" for prevention of sexually transmitted diseases using "inactive" pharmaceutical excipients.** *Biologicals* 1999, **27**:11-21
3. Gyotoku T, Aurelian L, Neurath AR: **Cellulose acetate phthalate (CAP): an 'inactive' pharmaceutical excipient with antiviral activity in the mouse model of genital herpesvirus infection.** *A ntivir Chem Chemother* 1999, **10**:327-332
4. Neurath AR, Li YY, Mandeville R, Richard L: **In vitro activity of a cellulose acetate phthalate topical cream against organisms associated with bacterial vaginosis.** *J Antimicrob Chemother* 2000, **45**:713-714
5. Manson KH, Wyand MS, Miller C, Neurath AR: **The effect of a cellulose acetate phthalate topical cream on vaginal transmission of SIV in rhesus monkeys.** *Antimicrob Agents Chemother* 2000, **44**:3199-3202
6. Berger EA, Doms RW, Fenyo EM, Korber BT, Littman DR, Moore JP, *et al*: **A new classification for HIV-1.** *Nature* 1998, **391**:240
7. Alkhatib G, Combadiére C, Broder CC, Feng Y, Kennedy PE, Murphy PM, *et al*: **CC CKR5: a RANTES, MIP-1α, MIP-1β receptor as a fusion cofactor for macrophage-tropic HIV-1.** *Science* 1996, **272**:1955-1958
8. Deng H, Liu R, Ellmeier W, Choe S, Unutmaz D, Burkhart M, *et al*: **Identification of a major co-receptor for primary isolates of HIV-1.** *Nature* 1996, **381**:661-666
9. Dragic T, Litwin V, Allaway GP, Martin SR, Huang Y, Nagashima KA, *et al*: **HIV-1 entry into CD4+ cells is mediated by the chemokine receptor CC-CKR-5.** *Nature* 1996, **381**:667-673
10. Feng Y, Broder CC, Kennedy PE, Berger EA: **HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor.** *Science* 1996, **272**:872-877
11. Trkola A, Dragic T, Arthos J, Binley JM, Olson WC, Allaway GP, *et al*: **CD4-dependent, antibody-sensitive interactions between HIV-1 and its co-receptor CCR-5.** *Nature* 1996, **384**:184-187
12. Wu L, Gerard NP, Wyatt R, Choe H, Parolin C, Ruffing N, *et al*: **CD4-induced interaction of primary HIV-1 gp120 glycoproteins with the chemokine receptor CCR-5.** *Nature* 1996, **384**:179-183
13. Kwong PD, Wyatt R, Robinson J, Sweet RW, Sodroski J, Hendrickson WA: **Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody.** *Nature* 1998, **393**:648-659
14. Littman DR: **Chemokine receptors: keys to AIDS pathogenesis?** *Cell* 1998, **93**:677-680
15. Rizzuto CD, Wyatt R, Hernandez-Ramos N, Sun Y, Kwong PD, Hendrickson WA, *et al*: **A conserved HIV gp120 glycoprotein structure involved in chemokine receptor binding.** *Science* 1998, **280**:1949-1953
16. Neurath AR, Strick N, Jiang S: **Synthetic peptides and anti-peptide antibodies as probes to study interdomain interactions involved in virus assembly: The envelope of the human immunodeficiency virus (HIV-1).** *Virol* 1992, **188**:1-13
17. Neurath AR, Debnath AK, Strick N, Li Y-Y, Lin K, Jiang S: **Blocking of CD4 cell receptors for the human immunodeficiency virus type 1 (HIV-1) by chemically modified bovine milk proteins: potential for AIDS prophylaxis.** *J Mol Recognition* 1995, **8**:304-316
18. Harada S, Koyanagi Y, Yamamoto N: **Infection of HTLV-III/LAV in HTLV-I-carrying cells T-2 and MT-4 and application in a plaque assay.** *Science* 1985, **229**:563-566
19. Neurath AR, Haberfield P, Joshi B, Hewlett IK, Strick N, Jiang S: **Rapid prescreening for antiviral agents against HIV-1 based on their inhibitory activity in site-directed immunoassays I. The V3 loop of gp120 as target.** *Antiviral Chem Chemother* 1991, **2**:303-312
20. Boyum A: **Isolation of lymphocytes, granulocytes and macrophages.** *Scand J Immunol* 1976, **Suppl. 5**:9-15
21. Neurath AR, Strick N: **Quantitation of cellulose acetate phthalate in biological fluids as a complex with ruthenium red.** *Anal Biochem* 2001, **288**:102-104
22. **QUANTA98.** *16 New England Executive Park, Burlington, MA 01803, USA, Molecular Simulations Inc*
23. Rumyantseva YI: **IR spectroscopic study of the structure of cellulose acetate phthalate and cellulose acetate succinate.** *Zhurnal Prikladnoi Spektroskopii* 1984, **41**:962-968

24. Peitsch MC: **ProMod and Swiss-Model: Internet-based tools for automated comparative protein modelling.** *Biochem Soc Trans* 1996, **24**:274-279
25. Hart TN, Ness SR, Read RJ: **Critical evaluation of the research docking program for the CASP2 challenge.** *Proteins* 1997, **Suppl 1**:205-209
26. Skinner MA, Ting R, Langlois AJ, Weinhold KJ, Lyerly HK, Javaherian K, *et al*: **Characteristics of a neutralizing monoclonal antibody to the HIV envelope glycoprotein.** *AIDS Res Hum Retroviruses* 1988, **4**:187-197
27. Matsushita S, Robert-Guroff M, Rusche J, Koito A, Hattori T, Hoshino H, *et al*: **Characterization of a human immunodeficiency virus neutralizing monoclonal antibody and mapping of the neutralizing epitope.** *J Virol* 1988, **62**:2107-2114
28. Burton DR, Pyati J, Koduri R, Sharp SJ, Thornton GB, Parren PWHI, *et al*: **Efficient neutralization of primary isolates of HIV-1 by a recombinant human monoclonal antibody.** *Science* 1994, **266**:1024-1027
29. Laal S, Zolla-Pazner S: **Epitopes of HIV-1 glycoproteins recognized by the human immune system.** *In Immunochemistry of AIDS, Chemical Immunology, Vol. 56. Edited by Norrby E. Basel: Karger;* 199391-111
30. Thali M, Moore JP, Furman C, Charles M, Ho DD, Robinson J, *et al*: **Characterization of conserved human immunodeficiency virus type 1 gp120 neutralization epitopes exposed upon gp120-CD4 binding.** *J Virol* 1993, **67**:3978-3988
31. Trkola A, Purtscher M, Muster T, Ballaun C, Buchacher A, Sullivan N, *et al*: **Human monoclonal antibody 2G12 defines a distinctive neutralization epitope on the gp120 glycoprotein of human immunodeficiency virus type 1.** *J Virol* 1996, **70**:1100-1108
32. Muster T, Steindl F, Purtscher M, Trkola A, Klima A, Himmler G, *et al*: **A conserved neutralizing epitope on gp41 of human immunodeficiency virus type 1.** *J Virol* 1993, **67**:6642-6647
33. Lapham CK, Ouyang J, Chandrasekhar B, Nguyen NY, Dimitrov DS, Golding H: **Evidence for cell-surface association between fusin and the CD4-gp120 complex in human cell lines.** *Science* 1996, **274**:602-605
34. Baba M, Pauwels R, Balzarini J, Arnout J, Desmyter J, De Clercq E: **Mechanism of inhibitory effect of dextran sulfate and heparin on replication of human immunideficiency virus in vitro.** *Proc Nati Acad Sci USA* 1988, **85**:6132-6136
35. Baba M, Schols D, Pauwels R, Nakashima H, De Clercq E: **Sulfated polysaccharides as potent inhibitors of HIV-induced syncytium formation: A new strategy towards AIDS chemotherapy.** *J AIDS* 1990, **3**:493-499
36. Maguire RA, Zacharopoulos VR, Phillips DM: **Carrageenan-based nonoxynol-9 spermicides for prevention of sexually transmitted infections.** *Sex Transm Dis* 1998, **25**:494-500
37. Javan CM, Gooderham NJ, Edwards RJ, Davies DS, Shaunak S: **Anti-HIV type 1 activity of sulfated derivatives of dextrin against primary viral isolates of HIV type 1 in lymphocytes and monocyte- derived macrophages.** *AIDS Res Hum Retroviruses* 1997, **13**:875-880
38. Shaunak S, Gooderham NJ, Edwards RJ, Payvandi N, Javan CM, Baggett N, *et al*: **Infection by HIV-1 blocked by binding of dextrin 2-sulphate to the cell surface of activated human peripheral blood mononuclear cells and cultured T-cells.** *Br J Pharmacol* 1994, **113**:151-158
39. Anderson RA, Zaneveld LJD, Usher TC: **Cellulose sulfate for use as antimicrobial and contraceptive agent.** *US Patent 6,063,773, issued May 16* 2000
40. Rusconi S, Moonis M, Merrill DP, Pallai PV, Neidhardt EA, Singh SK, *et al*: **Naphthalene sulfonate polymers with CD4-blocking and anti-human immunodeficiency virus type 1 activities.** *Antimicrob Agents Chemother* 1996, **40**:234-236
41. Neurath AR, Debnath AK, Strick N, Li YY, Lin K, Jiang S: **3-hydroxyphthaloyl-β-lactoglobulin. I. Optimization of production and comparison with other compounds considered for chemoprophylaxis of mucosally transmitted human immunodeficiency virus type 1.** *Antiviral Chem Chemother* 1997, **8**:131-139
42. Este JA, Schols D, de Vreese K, Van Laethem K, Vandamme A-M, Desmyter J, *et al*: **Development of resistance of human immunodeficiency virus type 1 to dextran sulfate associated with the emergence of specific mutations in the envelope gp120 glycoprotein.** *Mol Pharmacol* 1997, **52**:98-104
43. Moulard M, Lortat-Jacob H, Mondor I, Roca G, Wyatt R, Sodroski J, *et al*: **Selective interactions of polyanions with basic surfaces on human immunodeficiency virus type 1 gp120.** *J Virol* 2000, **74**:1948-1960
44. Greenhead P, Hayes P, Watts PS, Laing KG, Griffin GE, Shattock RJ: **Parameters of human immunodeficiency virus infection of human cervical tissue and inhibition by vaginal virucides.** *J Virol* 2000, **74**:5577-5586
45. Shattock RJ: **HIV infection of human cervical tissue in vitro and the effects of vaginal virucides.** *AIDS* 2001, **15 (Suppl. 1)**:S39
46. Choe H, Farzan M, Sun Y, Sullivan N, Rollins B, Ponath PD, *et al*: **The β-chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates.** *Cell* 1996, **85**:1135-1148
47. Cocchi F, DeVico AL, Garzino-Demo A, Cara A, Gallo RC, Lusso P: **The V3 domain of the HIV-1 gp120 envelope glycoprotein is critical for chemokine-mediated blockade of infection.** *Nature Med* 1996, **2**:1244-1247
48. Xiao L, Owen SM, Goldman I, Lal AA, deJong JJ, Goudsmit J, *et al*: **CCR5 coreceptor usage of non-syncytium-inducing primary HIV-1 is independent of phylogenetically distinct global HIV-1 isolates: delineation of consensus motif in the V3 domain that predicts CCR-5 usage.** *Virol* 1998, **240**:83-92
49. Speck RF, Wehrly K, Platt EJ, Atchison RE, Charo IF, Kabat D, *et al*: **Selective employment of chemokine receptors as human immunodeficiency virus type 1 coreceptors determined by individual amino acids within the envelope V3 loop.** *J Virol* 1997, **71**:7136-7139
50. Kawamura T, Cohen SS, Borris DL, Aquilino EA, Glushakova S, Margolis LB, *et al*: **Candidate microbicides block HIV-1 infection of human immature Langerhans cells within epithelial tissue explants.** *J Exp Med* 2000, **192**:1491-1500
51. Melander WR, Corradini D, Horvath C: **Salt-mediated retention of proteins in hydrophobic-interaction chromatography. Application of solvophobic theory.** *J Chromatogr* 1984, **317**:67-85
52. Javaherian K, Langlois AJ, LaRosa GJ, Profy AT, Bolognesi DP, Herlihy WC, *et al*: **Broadly neutralizing antibodies elicited by the hypervariable neutralizing determinant of HIV-1.** *Science* 1990, **250**:1590-1593
53. Bilello J, Belay S, Weislow 0: **Development of novel approaches to the evaluation of virucidal agents: separation of treated virions from test compounds.** *AIDS* 2001, **15 (Suppl. 1)**:S39
54. **SYBYL. 6.6.** *1699 Hanley Rd., St. Louis, MO 63144, Tripos Associates*