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Potent *In Vitro* Anti-Human Immunodeficiency Virus-1 Activity of Modified Human Serum Albumins

ROBERT W. JANSEN, GRIETJE MOLEMA, RUDI PAUWELS, DOMINIQUE SCHOLS, ERIK DE CLERCQ, and DIRK K. F. MEIJER

Department of Pharmacology and Therapeutics, University Centre for Pharmacy, Groningen University, Antonius Deusinglaan 2, Groningen, The Netherlands (R.W.J., G.M., D.K.F.M.), and Rega Institute for Medical Research, University of Leuven, B-3000 Leuven, Belgium (R.P., D.S., E.D.C.)

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SUMMARY

A series of neoglycoproteins was synthesized by coupling of thiophosgene-activated *p*-aminophenyl derivatives [*Biol. Cell.* **47**:95–110 (1983); *J. Histochem. Cytochem.* **32**:1091–1094 (1984)] of various sugars to human serum albumin. The compounds were evaluated for their *in vitro* activity against human immunodeficiency virus (HIV). Neoglycoproteins with the highest sugar content were found to be the most potent inhibitors of HIV-1-induced cytopathogenicity. However, this was not due to the nature of the sugar used but, rather, was related to the extra negative charge of the neoglycoproteins. To investigate whether the antiviral activity of the neoglycoproteins exhibited sugar specificity, increased with increasing negative charge, or depended on both sugar specificity and negative charge, we synthesized albumins and neoglycoproteins with an enhanced negative charge, by treatment with formaldehyde or succinic anhydride. Succinylated human serum albumin had the most pronounced net negative charge and had an IC_{50} of about 1 μg/ml. No cytotoxicity was observed at concentrations up to 1 mg/ml, implicating a selectivity index (CC_{50}/IC_{50}) of at least 10^3. To elucidate the mechanism of action of these anionic albumins, we investigated whether they interfered with HIV-1 adsorption to the cells, binding of anti-OKT4A monoclonal antibody (mAb) to the CD4 receptor, binding of anti-gp120 mAb to gp120, or inhibition of syncytium formation in co-cultures of HIV-1-infected HUT-78 cells with MOLT-4 cells. From these experiments, we conclude that albumins with an increased negative charge (a) are potent and nontoxic anti-HIV-1 agents, (b) cause a 50% reduction of syncytium formation in the same concentration range as their IC_{50} in the antiviral assay, and (c) do not bind to the OKT4A epitope of the CD4 receptor and only partly inhibit anti-gp120 mAb-gp120 interaction and virus-cell binding at concentrations that are 100 times higher than their IC_{50} in the antiviral assay. Therefore, we conclude that the modified albumins interfere with a post-binding event, of which one of the potential mechanisms is an interaction with the gp41 fusion protein, which is necessary for syncytium formation but is not involved in initial virus binding.

HIV, the causative agent of the acquired immunodeficiency syndrome and acquired immunodeficiency syndrome-related complex, shows selective tropism for lymphocytes and monocytes bearing the CD4 receptor on their cell surface (1–5). The high mannose-type *N*-linked glycans of the viral envelope gp120 are thought to play an important role in the interaction with the CD4 receptor (6–10). This specific event in the replicative cycle of HIV is the target for many antiviral agents. Polyanionic compounds such as Evans blue, suramin, and ATA were found to interfere with the virus-adsorption process (11–15). However, they are less potent in inhibiting virus adsorption than are the sulfated polysaccharides, which are among the most selective and potent anti-HIV agents described to date (16–22). A major drawback of many sulfated polysaccharides, however, is their anticoagulant activity and their unfavorable kinetic profile, which may limit their therapeutic usefulness. Mannose-binding lectins inhibit virus-induced cell fusion and *in vitro* infectivity through interactions with the viral envelope glycoprotein (6, 23, 24), suggesting that the high mannose-type oligosaccharide units of gp120 are essential for interaction with the CD4 molecule. In the process of developing carrier molecules for the specific delivery of antiviral agents to liver and blood cells (25–28), we made the interesting observation that some neoglycoproteins showed anti-HIV-1 activity. Therefore, we evaluated modified albumins, varying in negative charge, kind of sugar, and number of sugars/albumin molecule, for their anti-HIV-1 activity and their cytotoxicity. Furthermore, we determined the mechanism of anti-HIV-1 action of these

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ABBREVIATIONS: HIV, human immunodeficiency virus; HSA, human serum albumin; RPMI, RPMI 1640 medium; ATA, aurintricarboxylic acid; FPLC, fast protein liquid chromatography; PBS, phosphate-buffered saline; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; FITC, fluoroscein isothiocyanate; Suc-HSA, succinic anhydride-treated human serum albumin; mAb, monoclonal antibody.

compounds and investigated whether they interfere with the virus-adsorption process as general polyanions or in a sugar-specific way.

Materials and Methods

Chemicals. HSA (fraction V), *p*-aminophenyl sugar derivatives, and dextran sulfate (M_r 5000) were obtained from Sigma Chemical Co. (St. Louis, MO). Thiophosgene was obtained from Janssen Chimica (Beerse, Belgium). All other chemicals were of analytical grade or the best grade available and were used without further purification.

Synthesis of neoglycoproteins. Neoglycoproteins were prepared according to the methods of Monsigny *et al.* (29) and Kataoka *et al.* (30), with slight modifications. *p*-Aminophenyl-α-D-mannopyranoside (0.37 mmol) was dissolved in 20 ml of 80% ethanol, thiophosgene (2.1 mmol) was added, and the solution was stirred for 1.5 hr at room temperature. Nitrogen was bubbled through the solution for 2 hr to remove excess thiophosgene. Five milliliters of distilled water were added, the pH was adjusted to 6.0, and the solution was evaporated *in vacuo*. This "activated sugar" was redissolved in 10 ml of 0.1 M sodium carbonate buffer, pH 9.0, and 0.88 μmol of HSA dissolved in 20 ml of the same buffer was added. This solution was stirred at room temperature for 18 hr, with the pH being maintained at 9.0, and was purified on a Sephadex G25 column. Before lyophilization, the products were extensively washed with distilled water on a PM10 membrane in an Amicon stirred cell concentrator. The sugar content of the neoglycoproteins was determined using the phenol/sulfuric acid method (31); the amount of protein was determined both according to the method of Lowry *et al.* (32) and, using the protein assay kit of Bio-Rad, according to the method of Bradford (33).

Preparation of formaldehyde-treated albumin and Suc-HSA. Formaldehyde treatment was performed according to the method of Mego *et al.* (34). Briefly, HSA (500 mg) was dissolved in 50 ml of 0.2 M Na_2CO_3, pH 10.0, formaldehyde was added to a final concentration of 20%, and this solution was stirred for 72 hr in the dark at room temperature. The solution was filtered over a 0.2-μm filter to remove insoluble material, purified on a Sephadex G25 column, washed with distilled water on a PM10 membrane in an Amicon stirred cell concentrator, and finally lyophilized.

Succinylation of HSA was performed according to Ref. 35, with slight modifications; HSA (500 mg) was dissolved in 50 ml of 0.2 M K_2HPO_4, pH 8.0. Solid succinic anhydride (500 mg) was added, and the solution was stirred until all succinic anhydride was dissolved. The pH was kept between 8.0 and 8.5 with 6 M sodium hydroxide. Purification was performed as described for formaldehyde-treated HSA.

FPLC. The relative net negative charge of the modified albumins was determined on a FPLC system (Pharmacia, Woerden, The Netherlands) equipped with a Mono-Q anion exchange column (Pharmacia). Buffer A was a Tris·HCl buffer (0.02 M), pH 7.4, and buffer B consisted of buffer A plus 1 M NaCl (Fig. 2) or 2 M NaCl (Fig. 3). Elution was performed at a flow rate of 0.25 ml/min, with a gradient from 100% buffer A to 100% buffer B in 30 min. Samples were dissolved at a concentration of 1 mg/ml in buffer A, and 100 μl were injected into the FPLC system. The chromatographic behavior on a Superose-12 column (Pharmacia) was studied to investigate whether the modifications produced dimers or polymers.

Cells and virus. MT-4, a T_4 lymphocyte cell line carrying human T-cell lymphotropic virus (36), was used for the anti-HIV-1 assay and was kindly provided by Dr. N. Yamamoto, Yamaguchi University (Yamaguchi, Japan). The MT-4 cells were grown in RPMI, 1640 medium supplemented with 10% (v/v) heat-inactivated fetal calf serum and 20 μg/ml gentamycin. MOLT-4 cells (clone 8) (37) were used for the syncytium formation assay. The cells were maintained at 37° in a humidified atmosphere of 5% CO_2 in air. Every 3–4 days, cells were centrifuged and seeded at 2×10^5 cells/ml in new culture flasks. At regular time intervals, the cells were analyzed for the presence of *Mycoplasma* and were consistently found to be *Mycoplasma*-free.

HIV-1 (strain HTLV-III_B) (38) was obtained from the culture supernatant of persistently HIV-1-infected HUT-78 cells. The virus titer of the supernatant was determined in MT-4 cells. The virus stock was stored at −70° until used.

Antiviral assay. Antiviral activity of the test compounds was assessed by measurement of their inhibitory effects on virus-induced cytopathogenicity in MT-4 cells and was monitored by the MTT method, as described previously (39). The cytotoxicity of the compounds was also monitored by the MTT assay.

Syncytium formation assay. The modified albumins were diluted in RPMI and transferred to flat-bottomed 96-well microtiter plates (Falcon, Becton-Dickinson). Then, 5×10^4 HIV-1-infected HUT-78 cells, which were first washed twice to remove free virus particles, were added to the wells, immediately followed by the addition of 5×10^4 MOLT-4 cells to yield a final volume of 200 μl. The mixed cells were cultured at 37° in a CO_2 cell incubator. The first syncytia arose after 4–6 hr of co-cultivation. After 24 hr of co-cultivation, cells were analyzed by microscopic evaluation and laser flow cytofluorography, as described previously (40, 41).

Virus adsorption assay. The procedure for the detection of binding of HIV-1 particles to the cell surface has been described previously (42). Briefly, MT-4 cells were exposed to HIV-1 virions in the absence or presence of the test compounds. After incubation at 37° for 30 min, the cells were washed to remove unbound virus particles. Then, the cells were stained for indirect fluorescence, using a polyclonal antibody to HIV-1, and analyzed for HIV-1 particles bound to the cells, using laser flow cytofluorography.

CD4 immunofluorescence assay. CD4 expression was determined by FACSTAR (Becton-Dickinson) analysis, as described previously (43). Briefly, MT-4 cells were incubated for various times at room temperature in PBS, with or without test compound. The cells were then stained with optimal concentrations of the mAbs FITC-OKT4A (Ortho Diagnostics) or phycoerythrin-anti-Leu-3a and Simultest immune monitoring with control kit (FITC-labeled IgG1 and phycoerythrin-labeled IgG2) (Becton Dickinson) for 20 min at 4°, washed once in PBS, and fixed in 0.5 ml of 0.5% paraformaldehyde in PBS.

Glycoprotein gp120 immunofluorescence assay. Persistently HIV-1-infected HUT-78 cells (200,000 cells) in 100 μl of RPMI were washed twice in RPMI, incubated with the compounds at various concentrations at 20° for 15–20 min, washed twice in RPMI to remove residual compound, stained with anti-gp120 mAb 9284 (Dupont de Nemours, Brussels, Belgium) for 45 min at 37°, washed twice with RPMI, incubated with FITC-conjugated $F(ab')_2$ fragments of rabbit anti-mouse immunoglobulin antibody (Prosan, Ghent, Belgium) for 45 min at 37°, washed twice in PBS, resuspended in 0.5 ml of 0.5% paraformaldehyde in PBS, and analyzed by flow cytometry, as described previously (44).

Results

Anti-HIV-1 activity. By varying the sugar to protein ratio in the reaction mixture, we obtained neoglycoproteins with different amounts of incorporated sugars. We observed no polymers and no increase in dimers. The neoglycoproteins were evaluated for their anti-HIV-1 activity and cytotoxicity in the MT-4 cell culture system (Fig. 1 and Table 1). Only mannosylated albumins with a high sugar substitution, Man_{38}-HSA and Man_{40}-HSA, showed antiviral activity, with their IC_{50} values being 96 and 48 μg/ml, respectively. All other neoglycoproteins were inactive. At first sight, this suggests sugar specificity and a sugar substitution threshold for the intrinsic activity. However, it appeared that these mannosylated albumins also exhibited the most pronounced negative charge. Fig. 2 shows that the overall negative charge increases with increasing amounts of sugar coupled to HSA. We examined whether the observed antiviral activity was caused by the mannose groups, the en-

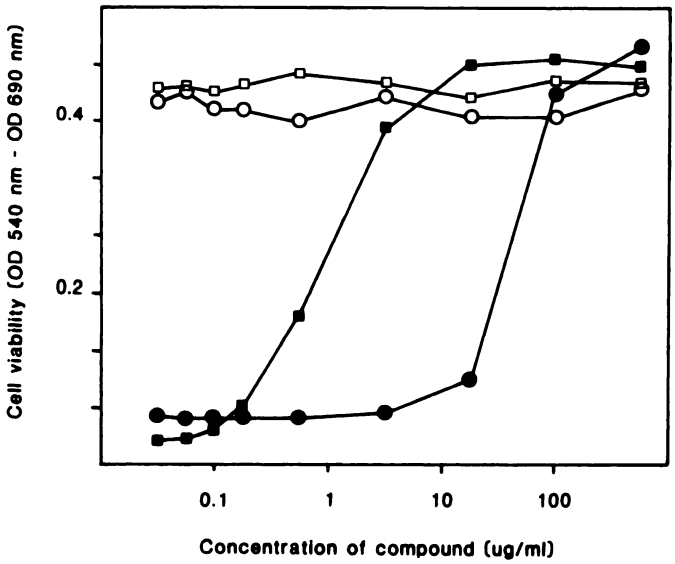

Fig. 1. Typical dose-response curves of Man_{36}-HSA (*circles*) and Suc-HSA (*squares*). *Closed symbols*, cell viability in the presence of virus and different concentrations of compound. *Open symbols*, cell viability in the presence of different concentrations of compound.

TABLE 1

Inhibitory effects of the modified albumins on HIV-1-induced cytopathogenicity and giant cell formation

All data represent mean values for at least three separate experiments.

Compound	CC_{50}[a]	IC_{50}	
		Replication[b]	Syncytium assay[c]
	μg/ml	μg/ml	
HSA	>500	>500	>500
Man_7-HSA	>500	>500	>500
Man_{22}-HSA	>500	>500	>500
Man_{36}-HSA	>500	96.0	>500
Man_{40}-HSA	>500	48.5	100.0
Fuc_{10}-HSA	>500	>500	>500
Fuc_{25}-HSA	>500	>500	>500
Glu_5-HSA	>500	>500	>500
Glu_{25}-HSA	>500	>500	>500
Gal_5-HSA	>500	>500	>500
Gal_{32}-HSA	>500	>500	>500
Suc-HSA	>500	1.0	2.0
Suc-Man_7-HSA	>500	1.8	10.0
Suc-Man_{25}-HSA	>500	4.8	15.0
Suc-Man_{40}-HSA	>500	2.8	10.0
Suc-Fuc_{10}-HSA	>500	0.6	1.0
Suc-Fuc_{25}-HSA	>500	11.2	15.0
Suc-Glu_5-HSA	>500	1.3	2.0
Suc-Glu_{25}-HSA	>500	1.6	5.0
Suc-Gal_5-HSA	>500	1.6	10.0
Suc-Gal_{32}-HSA	>500	9.5	20.0
Dextran sulfate (M_r 5000)	>500	0.6	28.0

[a] 50% cytotoxic concentration, based on the reduction of the viability of mock-infected MT-4 cells.

[b] 50% inhibitory concentration, based on the inhibition of HIV-1-induced cytopathogenicity in MT-4 cells.

[c] 50% inhibitory concentration, based on the inhibition of syncytium formation upon co-culturing of persistently HIV-1-infected HUT-78 cells with MOLT-4 cells.

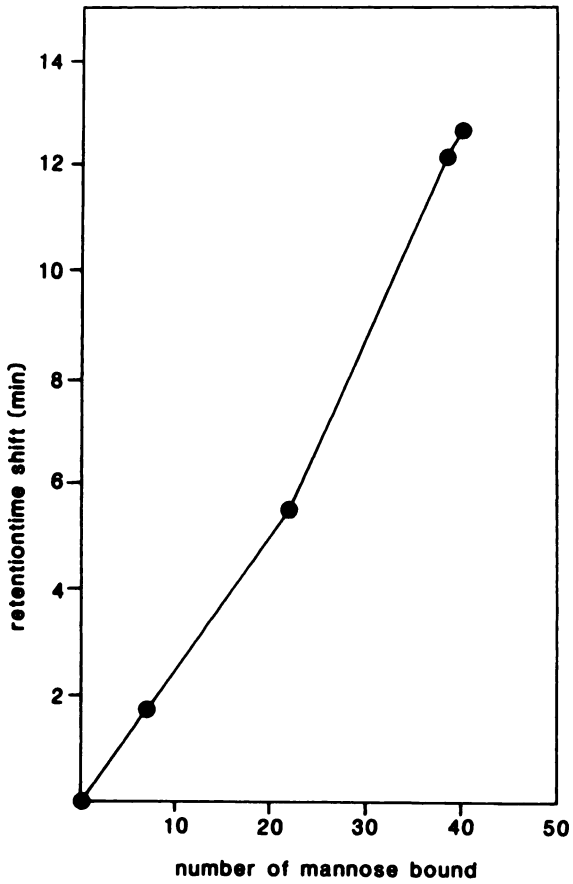

Fig. 2. Relation between the number of (para-aminophenyl-)mannose residues bound to albumin and net negative charge. The retention time shift was calculated by substraction of the retention time, on a Mono-Q anion exchange column, of the parent albumin (19.8 min) from the observed retention times of the modified albumins.

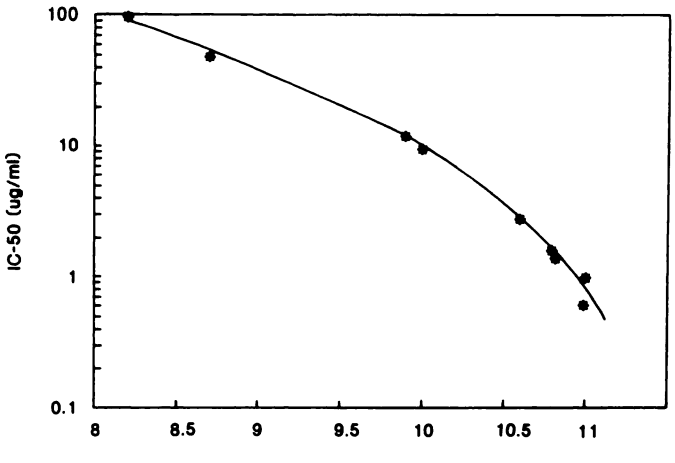

Fig. 3. Relation between the negative charge of modified albumins and their anti-HIV-1 activity. The relative negative charge was determined using a Mono-Q anion exchange column. The values represent the additional retention time (min), compared with the parent HSA.

hanced negative charge, or a combination of the two. Fig. 3 shows that there is a clear correlation between antiviral activity and negative charge, and it seems that the carbohydrate portion of the neoglycoprotein does not contribute to this antiviral activity (Table 1). Moreover, simple succinylation of the lysine ϵ-NH_2 of albumin resulted in the most anionic compound with the lowest IC_{50} (1 μg/ml). Furthermore, increasing the negative charge of the neoglycoproteins by coupling with FITC or by treatment with formaldehyde improved the antiviral activity (data not shown). All modified albumins were not cytotoxic at concentrations of up to 1000 μg/ml. This resulted in selectivity

indices well above 1000. It has now been well established that anionic substances like the sulfated polysaccharides (e.g., dextran sulfate) owe their anti-HIV activity to interference with the virus-adsorption process and, at higher concentrations, to the inhibition of multinucleated giant cell formation. We investigated whether the modified HSAs have a similar mode of action.

Virus adsorption inhibition. The modified albumins were tested for their ability to inhibit binding of virus particles to MT-4 cells. Dextran sulfate was used as a positive control. Dextran sulfate almost completely inhibited virus adsorption at a concentration of 25 μg/ml, whereas at a concentration of 100 μg/ml the modified albumins only partly inhibited this process (Table 2).

Syncytium formation inhibition. As can be seen from Table 1, the neoglycoproteins were also inhibitory to HIV-1-induced syncytium formation, at IC_{50} values similar to those that were inhibitory to HIV-1 replication. Some modified albumins (Suc-HSA, Suc-Fuc_{10}-HSA, and Suc-Glu_5-HSA) caused a 50% inhibition of syncytium formation at a concentration as low as 2 μg/ml. In contrast, the concentration of dextran sulfate needed to give a 50% reduction in syncytium formation was 28 μg/ml, which is about 50 times higher than its IC_{50} in the antiviral assay (0.6 μg/ml).

OKT4A mAb-CD4 inhibition. OKT4A mAb binds to an epitope of the CD4 molecule responsible for HIV adsorption and is able to prevent binding of HIV particles to the cells (45, 46). ATA interacts specifically with this epitope of the CD4 molecule (43) and was used as a positive control. ATA completely inhibited OKT4A mAb-CD4 interaction at a concentration of 25 μg/ml. The modified albumins and dextran sulfate did not interfere with this interaction at a concentration as high as 100 μg/ml, indicating that they do not bind to this epitope of the CD4 receptor.

Anti-gp120 mAb-gp120 inhibition. We also examined whether the modified albumins directly interact with the viral gp120 glycoprotein. Therefore, we used persistently HIV-1-infected HUT-78 cells (more than 90% $gp120^+$) and a specific anti-gp120 mAb recognizing the V3 region of gp120, which plays an important role in giant cell formation (47). Dextran sulfate inhibited binding of anti-gp120 mAb to gp120 in a concentration-dependent way (44) and in a range similar to its antiviral activity. The modified albumins also inhibited anti-gp120 mAb-gp120 interaction in a concentration-dependent manner (Table 3). However, the concentration needed for 50% inhibition was about 100-fold higher than the IC_{50}, suggesting that "shielding" of gp120 is unlikely to be the sole mechanism of action of these compounds.

TABLE 2

Inhibitory indices for virus binding (II_{vb}) of the modified albumins to MT-4 cells

The inhibitory index for virus binding (II_{vb}) (41) was calculated according to the following formula: $II_{vb} = 1 - (MF_{VC} - MF_{CC})/(MF_V - MF_C)$, whereby MF_{VC} is the mean fluorescence with a given concentration of the compound in HIV-I-inoculated cells, MF_{CC} is the mean fluorescence for the control cells (not exposed to HIV-I) treated with compound, MF_V is the mean fluorescence for the HIV-I-inoculated cells (not treated with any compound), and MF_C is the mean fluorescence for the control cells (not exposed to HIV-I and not treated with any compound). If $II_{vb} = 1$, there is total inhibition of virus binding; if $II_{vb} = 0$, there is no inhibition of virus binding. All data are the mean values for at least two separate experiments.

Compound	Concentration	II_{vb}
	μg/ml	
Man_{40}-HSA	100	0.26
Fuc_{25}-HSA	100	0.22
Suc-HSA	100	0.60
Suc-Man_7-HSA	100	0.32
Suc-Man_{25}-HSA	100	0.26
Suc-Man_{40}-HSA	100	0.33
Suc-Fuc_{10}-HSA	100	0.46
Suc-Glu_{25}-HSA	100	0.60
Dextran sulfate (M_r 5000)	25	0.92

Discussion

Several authors have pointed out that the oligosaccharide part, especially the high mannose-type *N*-linked glycans, of gp120 is essential for the high affinity binding of HIV-1 to the CD4 molecule (6, 8, 9, 23, 24). Our observation that the neoglycoproteins Man_{38}-HSA and Man_{40}-HSA were able to protect MT-4 cells from HIV-1-induced cytopathogenicity gave rise to the question whether this activity was based on a specific interference of the HSA-bound mannose groups with gp120-CD4 interactions. Preliminary data suggest that the modified albumins do not exert antiviral activity at an intracellular level. After a dose of 50 pmol of ^{125}I-labeled modified albumin, less then 0.16% of the dose was associated per 10^6 freshly isolated peripheral blood lymphocytes. The amount of cell-associated radioactivity at 4° and 37° did not differ significantly and was constant over time, indicating that no substantial uptake occured.

In the present study, we have shown that modification of ε-

TABLE 3

Effects of the compounds on the binding of anti-gp120 mAb to persistently HIV-I-infected HUT-78 cells

HIV-1-infected HUT-78 cells (200,000/100 μl of RPMI) were washed twice, incubated with the compounds at the indicated concentrations at 20° for 15 min, stained, and analyzed as described in Material and Methods. The inhibitory index for anti-gp120 mAb binding inhibition (II_{gp120}) was calculated according to the formula: $II_{gp120} = 1 - (MF_{gp120x} - MF_c/MF_{gp120} - MF_c)$, whereby MF_{gp120} is the mean fluorescence for the cells incubated with anti-gp120 mAb, MF_{gp120x} is the mean fluorescence for the cells incubated with the test compound and anti-gp120 mAb, and MF_c is the mean fluorescence of the cells incubated with FITC-labeled rabbit anti-mouse IgG F(ab′)$_2$.

Compound	Concentration	II_{gp120}
	μg/ml	
Fuc_{25}-HSA	100.00	0.00
Man_{40}-HSA	100.00	0.23
	20.00	0.09
	4.00	0.06
	0.80	0.07
	0.16	0.04
Suc-Man_{40}-HSA	100.00	0.77
	20.00	0.31
	4.00	0.07
	0.80	0.03
	0.16	0.01
Suc-Gal_5-HSA	100.00	0.79
	20.00	0.41
	4.00	0.10
	0.80	0.04
	0.16	0.01
Suc-Fuc_{10}-HSA	100.00	0.80
	20.00	0.41
	4.00	0.09
	0.80	0.03
	0.16	0.00
Dextran sulfate (M_r 5000)	100.00	1.00
	20.00	0.99
	4.00	0.88
	0.80	0.42
	0.16	0.26

NH_2-lysine residues of HSA with *p*-aminophenyl sugars or succinic anhydride renders this protein highly anionic and that the anti-HIV-1 activity is correlated with this negative charge. Suc-HSA and Suc-Fuc_{10}-HSA are the most anionic albumins tested and have IC_{50} values as low as 1.0 and 0.6 μg/ml, respectively. No evidence was found that the sugar portion actually contributed to the antiviral activity.

In this study, we tried to elucidate the mechanism of action of the modified albumins and we compared their mode of action with that of another polyanion, the sulfated polysaccharide dextran sulfate (IC_{50}, 0.6 μg/ml). As can be seen from Table 2, both dextran sulfate and the modified albumins inhibited the adsorption of virus to MT-4 cells, although the latter required much higher concentrations. To achieve a 50% reduction of syncytium formation (Table 1), a concentration of 28 μg/ml dextran sulfate (about 50 times its IC_{50} in the antiviral assay) was needed. In contrast, the modified albumins gave a 50% reduction of syncytium formation in the same concentration range as their IC_{50} (2 μg/ml). Syncytium formation is supposed to be one of the major causes of T_4 cell depletion (40, 48), and a potent inhibitor of this process might be of great therapeutic value.

One of the differences between the virus adsorption assay and the syncytium assay is that in the latter, apart from interferences with gp120-CD4 interactions, interference with the fusion process of gp41 with the cell membrane might be revealed.

The results described above led to the hypothesis that the modified albumins might have a mode of action different from that of dextran sulfate. Neither dextran sulfate (42) nor the modified albumins inhibited binding of OKT4A mAb to the MT-4 cell at concentrations up to 100 μg/ml (data not shown). Therefore, they do not bind directly to the OKT4A epitope of the CD4 molecule.

It was recently reported that dextran sulfate interacts specifically with gp120 (44) and that this "shielding" of gp120 may be the major mode of action of the sulfated polysaccharides. These agents inhibit binding of anti-gp120 mAb to gp120 in a concentration-dependent manner and at concentrations similar to that needed for their antiviral activity. In contrast, the modified albumins only partly inhibited this process at concentrations that are 100 times higher than their IC_{50} in the antiviral assay. Although all the described assays were conducted under different conditions and cannot be directly compared, the differences in the concentration of modified albumins required to inhibit virus replication and anti-gp120 mAb-gp120 binding are large enough to suggest that direct binding to gp120 is unlikely to be the only mechanism of antiviral action. The albumins may disturb events involving gp41 or gp120/gp41 interactions distinct from initial binding of virus to the cells but essential for syncytium formation and virus replication. Until now, substances interfering with these events have not been described. Interesting, however, is the observation that new mannose-specific binding lectins totally inhibited HIV infection and HIV-induced cell fusion at a concentration of 0.5–5 μg/ml (48). These plant lectins did not inhibit HIV binding to $CD4^+$ cells, suggesting that these lectins, like the modified albumins, interact with the virus cell membrane fusion process. Although the extreme modifications of albumin produced in our test tubes will not occur *in vivo*, the existence of negatively charged albumins in the bloodstream has been described (49–52). It remains to be established whether modified albumins that are produced under physiological or pathological conditions have major influences on some stages of HIV replication.

Preliminary data on the kinetic profiles of Suc-HSA in intact rats and in the isolated perfused rat liver indicate that Suc-HSA is removed from the circulation with a high affinity but low capacity uptake mechanism in nonparenchymal liver cells. Therefore, constant plasma levels well above the IC_{50} of Suc-HSA could be obtained.

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Send reprint requests to: Robert W. Jansen, Department of Pharmacology and Therapeutics, University Centre for Pharmacy, Groningen University, Antonius Deusinglaan 2, 9713 AW Groningen, The Netherlands.