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Sulfated β-Glucan Derived from Oat Bran with Potent Anti-HIV Activity

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China is a major producer of oats; the annual harvested area of 350,000 ha yields approximately 465,000 tons, giving an average yield of 1.33 tons/ha. The bran is not used for animal feed as it is of poor digestibility and low nutritive content and is considered a waste byproduct. Therefore, it is advantageous to produce a value-added product from the bran. We extracted the native polysaccharide, a linear (1–3)-, (1–4)-linked β-glucan (OBG) from the oat bran and synthesized a sulfated derivative OBGS containing 36.5% sulfate. OBGS had potent activity against a primary isolate of human immunodeficiency virus (HIV-1) in peripheral blood mononuclear cells at a concentration (EC_{50} = 5.98 × 10^{-4} μM) approximately 15,000 times below its cytotoxic concentration. OBGS was also active postinfection (EC_{50} = 5.3 × 10^{-4} μM) and protected pretreated peripheral mononuclear cells (EC_{50} = 5.2 × 10^{-2} μM) washed free of the compounds prior to infection. Thus, OBGS has potential as a vaginal microbicide and is the first such report for oat bran derived sulfated β-glucan.

KEYWORDS: ***Avena sativa*** **L.; oat bran sulfated β-glucan; potent anti-HIV activity; low cytotoxicity**

INTRODUCTION

China is one of the world's major producers of oats (*Avena sativa* (*A. sativa*)). Oats are cultivated in 18 provinces and regions situated mainly in the cooler, moist areas of northern China, for which other crops such as maize and soybean are less well adapted (*1, 2*). The annual harvested area of 350,000 ha yields approximately 465,000 tons, giving an average yield of 1.33 tons/ha (*3*). More than 50% is used for animal fodder (kernel, hay, silage, and grazing), approximately 40% is for human consumption and industrial use, and 6–7% is used as seed (*2*). Two major types of oat are cultivated: hexaploid hulled oats, *A. sativa*, and the naked variety, *A. sativa* L., sometimes referred to as *Avena nuda* (*A. nuda*) so-called because the kernel readily threshes free of the hulls (bran). The kernels can be fed to young animals without further processing, but the bran is not suitable as it is of low digestibility and energy content, so a significant proportion is discarded. However, oat bran is a valuable source of β-glucans (*4*) and is approved by the FDA as food supplements for those with coronary heart disease (*5*). β-Glucans also have beneficial properties including physiological, pharmaceutical, antimicrobial, and antitumor properties (*6*).

There has been a good deal of recent interest in the chemical modification of glucans. For example, sulfation increases solubility and enhances biological activities (*7*) including immunomodulatory, antitumor (*8*), and antiviral activity (*9–11*). Thus further development of a value-added oat bran β-glucan as a compound with health and medicinal value is certainly warranted.

We were particularly interested in the potential of sulfated oat bran β-glucan as a vaginal microbicide for use in resource constrained settings that would reduce HIV-l transmission by preventing entry of the virus to the cell. Several studies have shown that sulfated β-glucans possess properties that make them suitable for formulation as virucidal gels; they inhibit HIV-1 infection at micromolar concentrations, syncytium (multinucleated giant cell) formation, and cell to cell adhesions (*12–14*), they are not cytotoxic, and, of great importance, selection for drug resistant HIV-1mutants is very low in vitro (*14, 15*).

Thus we extracted the native oat bran glucan (OBG) of *A. sativa* L., originating from Hebei province, China and prepared a sulfated derivative (OBGS). We then examined their anti-HIV-1 properties using a clinical isolate of HIV-1 in peripheral blood mononuclear cell (PBMCs (see Abbreviations Used)) cultures. PBMCs contain CD4-expressing T-cells, subsets of which are targeted by HIV-1 in vivo. To the best of our knowledge, this is the first report on anti-HIV-1 activity of an oat bran derived sulfated glucan.

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Table 1. Molecular Weight

compound ID[a]	M_r[b]
OBG	5.0×10^5
OBGS	6.86×10^5
DS	5×10^4

[a] OBG is oat bran glucan, OBGS is the sulfated derivative, and DS is dextran sulfate 5000. [b] M_r is molecular weight.

MATERIALS AND METHODS

Extraction of the Native Polysaccharide from Oat Bran. Air-dried oat bran (1 kg) from the naked oat variety, *A. sativa* L., was defatted with 95% alcohol at room temperature (RT) and the residue extracted in boiling water for 3 h (3 × 20 L). The merged extract was then concentrated to 20 L under reduced pressure and deproteinized with trichloroacetic acid. After centrifugation, the supernatant was dialyzed against tap water for 2 days and distilled water (1 day). The dialysate was then concentrated under reduced pressure to 20 L, after which 20 L of 95% alcohol was added slowly at RT with stirring. This mixture was stored overnight, and the precipitate was collected, redissolved in distilled water, and lyophilized to give the glucan designated OBG.

Preparation of Sulfated Glucan. A 2 g amount of OBG was suspended in 40 mL of dry DMF and stirred for 12 h at RT and then 10 mL freshly prepared chlorosulfonic−pyridine complex added as previously reported (*16*). The sulfation reaction was carried out with stirring under N_2 at 60 °C for 4 h, after which 5% NaOH was added to neutralize the mixture. The sulfated glucan was precipitated with cold ethanol, redissolved in water, dialyzed against water for 2 days, and lyophilized to give a sulfated glucan designated OBGS.

Sugar Composition of OBG. A 5 mg amount of OBG was hydrolyzed with 2 mol/L trifluoroacetic acid (aqueous) at 120 °C for 2 h. The mixture was then divided into two parts; one part was analyzed by TLC using glucose as standard, and the other part was reduced by $NaBH_4$ and converted into the corresponding alditol acetate as previously described (*17*).

Methylation Analysis. OBG and OBGS were methylated 3 times according to the method of Needs and Selvendran (*18*) using NaOH powder and methyl iodide in dimethyl sulfoxide. The per-methylated product was hydrolyzed with 2 mol/L TFA (120 °C, 2 h), reduced, and acetylated. The partially methylated alditol acetates were analyzed by GC-MS, and the temperature program was isothermal at 140 °C for 3 min followed by a 3 °C/min gradient up to 250 °C.

The sulfate content of OBGS was determined as previously described (*19*), and the molecular weights of OBG and OBGS were obtained according to the method of Wei and Fang (*20*). IR spectra were obtained from a Perkin-Elmer 2000 IR spectrometer(Perkin-Elmer, Fremont, CA).

Virus and Cells. HIV-1_{PE106} (PE106, subtype B, X4), a primary isolate that had undergone a single passage in PBMCs in which it induces syncytia (*21*), was used to evaluate the antiviral activity of OBG and OBGS. Virus stocks were prepared in CD8-depleted PBMCs previously stimulated with PHA (5 μg/mL, Sigma) for 3 days as previously described (*22*). PBMCs were maintained in growth medium (Panserin mix, a medium composed of 45% RPMI, 45% serum free medium [PanSerin 501, Pan Biotech GmbH, Germany], 10% heat-inactivated FBS, pen/strep 1000/2000 units (U)/mL, and recombinant human interleukin 2, rhIL2, Roche Products, 40 U/mL).

Anti-HIV Activity Evaluated by Four Methods. *1. Compounds Added at the Time of Infection.* OBG and OBGS were dissolved in distilled water and serial log dilutions (1 to 0.00001 μM) were prepared in growth medium. Antiviral activity was evaluated in CD8-depleted PBMCs at 7 days postinfection (dpi) as previously described (*23*). Dextran sulfate 5000 (DS; Pfizer, U.K.) which inhibits HIV-1 replication in vitro (*13*) was included as a positive control. Briefly, a 10 or 100 predetermined tissue culture infective $dose_{50}$ ($TCID_{50}$) of PE106 was added to serial log diluted compounds in triplicate at RT in 96-well flat-bottomed plates (Becton Dickinson, U.K.) prior to the addition of PBMCs as previously described (*23*). Plates were then incubated for 2 h at 37 °C, 5% CO_2 for virus adsorption, washed extensively to remove the compounds and unadsorbed virus, and resuspended in growth medium. At 7 dpi the concentration of the virus core protein, p24 (a measure of virus replication) in cell free supernatants, was determined by p24 ELISA (Innogenetics, Gent, Belgium) according to the manufacturer's protocol. The percent inhibition of p24 production was calculated according to the formula, $(A - B)/A \times 100$, where A is the mean p24 concentration of the virus control (untreated infected cells) and B, the mean p24 concentration of test wells (infected, treated cells).

2. Compounds Added Postinfection. To determine if the compounds inhibited virus replication at steps post-binding/cell entry as reported for DS (*13, 23*), we looked at postinfection antiviral activity using the method described above except that (i) the compounds were added to 2 h, PE106-infected cells that had been washed free of unadsorbed virus 3 times, and (ii) infected-cells were cultivated with the compounds for 7 days before evaluating p24 inhibition.

3. Compounds Added to Cells Preinfection. To determine if the mechanism of HIV inhibition involved compounds binding to/interacting with the cell surface, thus preventing virus entry, we looked at antiviral activity in pretreated PBMCs that were washed free of the compounds prior to HIV infection. Thus PBMCs were incubated with log diluted compound for 1 h, washed free of the compound (×3) and infected in triplicate as before. Infected cells were then washed free of unadsorbed virus and p24 inhibition was again evaluated at 7 dpi. For each assay, the dilution required to inhibit p24 production by 50% (EC_{50}) and 90% (EC_{90}) was calculated from graphs of percentage p24 inhibition against compound concentration. Results are shown as the mean of three to six replicates together with the standard deviation.

4. Syncytium Inhibition. Infected PBMC cultures were examined microscopically for inhibition of HIV-1-induced syncytia at 7 dpi.

Cytotoxicity. Cytotoxicity was evaluated in mock infected CD8-depleted PBMCs incubated in replicates of six with serial dilutions (10–0.0001 μM) of the compounds for 7 days. The viability of treated PBMCs was compared with that of untreated cell controls cultured under identical conditions using the Guava PCA-96 cell analyzer (Guava Technologies Inc., Hayward, CA) and the Guava ViaCount flex reagent (a vital stain) as described by Wang et al. (*23*). Results for 10,000 cells/well were analyzed using the Guava ViaCount Easyfit analysis algorithm and are given as the absolute count of viable cells per milliliter.

The dilution required to kill 50% uninfected PBMCs (CC_{50}) was derived from graphs of viable cell numbers per milliliter against compound dilution. Results are given as the calculated mean of six replicates together with the standard deviation (SD). The selectivity index (SI) was derived from the CC_{50}/EC_{50}

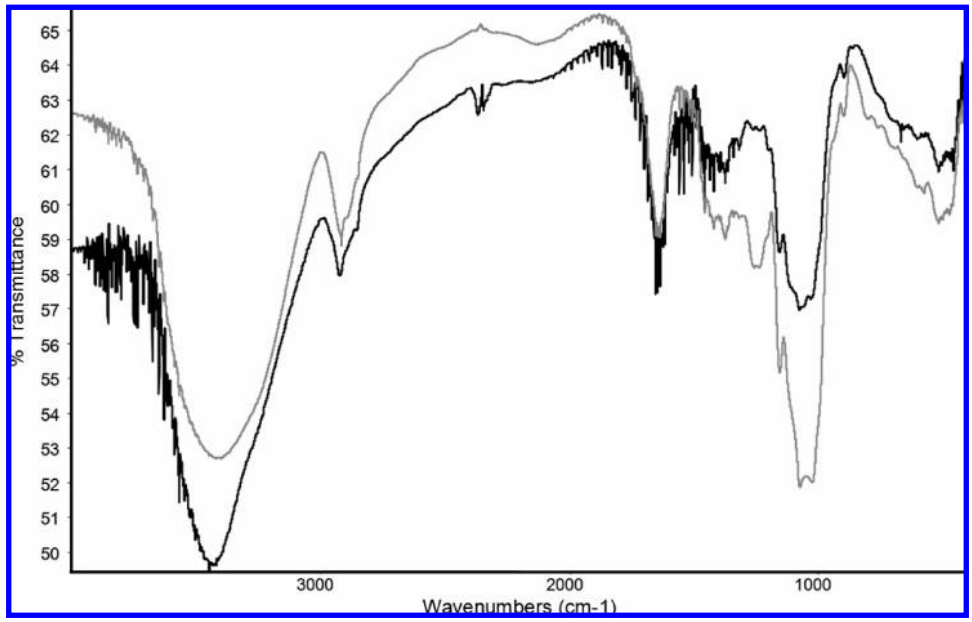

Figure 1. IR spectra of native OBG (black line) and its sulfated derivative OBGS (gray line) in the 400–4000 cm^{-1} region. OBG exhibits two strong absorption bands at 3121 and 1073 cm^{-1}, associated with O−H and C−O bonding, respectively. Absorption at 890 cm^{-1} indicates the β configuration. The new band at 1269 cm^{-1} for OBGS results from vibrations of S═O groups.

Table 2. Methylation Analysis of OBG and OBGS[a]

alditol acetates	OBG[b]	OBGS[b]	deduced linkage
2,3,4,6-tetramethylglucose	trace	trace	Glc(1→
2,4,6-trimethylglucose	1	NP[c]	→3)Glc(1→
2,3,6-trimethylglucose	3	NP	→4)Glc(1→
2,4-dimethylglucose	NP	1	→3,6)Glc(1→
2,3-dimethylglucose	NP	3	→4,6)Glc(1→

[a] The native oat bran glucan (OBG) and its sulfated derivative (OBGS) were methylated (×3) as previously described (*18*) using NaOH and methyl iodide in dimethyl sulfoxide, hydrolyzed with 2 mol/L TFA, reduced, acetylated, and the partially methylated alditol acetates analyzed by GC-MS. [b] Ratios are given. [c] NP means not present.

RESULTS

Structural Characterization. A 57 g amount of native glucan (OBG) was obtained from 1 kg of oat bran. The sugar composition analysis showed OBG to be composed of glucose only. Methylation analysis showed that the OBG contained mainly 2,4,6-trimethylglucosyl residues and 2,3,6-trimethylglucosyl residues in a ratio of 1:3, indicating it to be a linear (1–3)-linked glucan with (1–4)-linked backbone.

A 1.6 g amount of OBGS was prepared by the sulfation reaction, and subsequent analysis showed that it contained 36.5% sulfate. Methylation analysis of OBGS showed the presence of 2,4-dimethylglucosyl and 2,3-dimethylglucosyl residues in a ratio of 1:3, indicating that the sulfate group substituted at position O-6. The molecular weights of OBG and OBGS are given in **Table 1**.

The sulfation of oat bran glucan was confirmed by IR. **Figure 1** shows the IR spectra of native OBG and its sulfated derivative OBGS in the 400–4000 cm^{-1} region. OBG exhibited two strong absorption bands at 3121 and 1073 cm^{-1}, which were associated with O−H and C−O bonding, respectively. The absorption at 890 cm^{-1} indicated that the polysaccharide was in a β configuration. A new band at the region of 1269 cm^{-1} was observed for OBGS as a result of vibrations of (S═O) groups, indicating the successful sulfation of OBG.

Anti-HIV-1 Activity. We compared the anti-HIV-1 activity of OBG with that of OBGS using DS as a positive control (*13*). OBG showed little or no inhibition of HIV p24 production when added at the time of infection, 2 h postinfection or following pretreatment of PBMCs, and is not discussed further (**Tables 3** and **4**). In contrast, the sulfated derivative showed potent antiviral activity that was dependent on the virus challenge. Following the lower virus challenge (10 $TCID_{50}$) the concentration required to inhibit PE106 p24 production by 50% (EC_{50} = 7.86 $\times 10^{-5}$ μM) was approximately half that of DS (EC_{50} = 1.67 $\times 10^{-4}$ μM, **Table 3**), and the concentration required to inhibit p24 production by 90% was 2.3 $\times 10^{-2}$ μM. At a higher HIV-1 challenge (100 $TCID_{50}$) the EC_{50} and EC_{90} increased to 5.98 $\times 10^{-4}$ and 5.0 $\times 10^{-2}$ μM, respectively (**Table 3**). Thus sulfation of OBG had a significant effect on anti-HIV-1 activity demonstrating potent inhibition of p24 production at nanomolar concentrations.

OBGS showed comparable anti-HIV-1 activity versus the higher virus challenge (100 $TCID_{50}$) when added 2 h postinfection (**Table 4**; EC_{50} = 5.3 $\times 10^{-4}$ μM; EC_{90} = 5.3 $\times 10^{-2}$ μM) as previously reported with DS (*13, 23*). It is likely that postinfection activity involves inhibition of early postadsorption steps such as virus internalization and inhibition of cell-to-cell transmission in successive cycles of replication. OBGS (and DS) may also interact with the cell surface via the negatively charged sulfate groups, effectively reducing virus binding to the CD4 and or co-receptor sites required for cell entry. To investigate this, PBMCs were pretreated with serial 10-fold dilutions of the compounds for 1 h and washed extensively prior to addition of a high virus challenge (100 $TCID_{50}$ of PE106). Inhibition of p24 production was again determined at 7 dpi, but antiviral activity was significantly reduced with OBGS (EC_{50} = 5.2 $\times 10^{-2}$ μM; EC_{90} = 0.75 μM), and for DS, antiviral activity was lost. These results suggest that OBGS interacts with the cell surface but that binding to the virus surface is required for potent antiviral activity.

Microscopic examination of PE106-infected cultures showed that the concentration of OBGS or DS required to inhibit 50% syncytium formation (not shown) was approximately 10-fold

Table 3. Anti-HIV Activity of Sulfated Oat Bran β-Glucan[a]

	EC_{50} [b] (μM)/$TCID_{50}$		EC_{90} [c] (μM)/$TCID_{50}$			
compound	10	100	10	100	CC_{50}[d] (μM)	SI[e]
OBGS	$7.86 \times 10^{-5} \pm 7.5 \times 10^{-6}$	$5.98 \times 10^{-4} \pm 3.50 \times 10^{-5}$	$2.3 \times 10^{-2} \pm 1.3 \times 10^{-3}$	$5.0 \times 10^{-2} \pm 5.0 \times 10^{-3}$	$>9 \pm 0.44$	>15050
DS	$1.67 \times 10^{-4} \pm 3.1 \times 10^{-5}$	$8.33 \times 10^{-4} \pm 3.118 \times 10^{-5}$	$2.8 \times 10^{-2} \pm 9.0 \times 10^{-3}$	$6.5 \times 10^{-2} \pm 3.6 \times 10^{-3}$	7.67 ± 0.69	9232

[a] 10 or 100 tissue culture infectious doses ($TCID_{50}$) of HIV-1_{PE106} were added to serial 10-fold dilutions of native oat bran glucan (OBG), its sulfated derivative (OBGS), and dextran sulfate (DS) prior to the addition of CD8-depleted PBMCs (peripheral blood mononuclear cells). Results show the mean ± SD from three to six replicates. [b] EC_{50} is the concentration required to inhibit HIV p24 antigen production by 50%. [c] EC_{90} the concentration required to inhibit HIV p24 antigen production by 90% 7 days postinfection. [d] CC_{50} is a measure of cytotoxicity being the concentration required to kill 50% uninfected cells. [e] SI is the selective index derived from the CC_{50}/EC_{50} for a virus challenge of 100 $TCID_{50}$.

Table 4. Pre- and Postinfection Inhibition of Sulfated Oat Bran β-Glucan[a]

	preinfection inhibition[b]		postinfection inhibition[c]	
compound	EC_{50} (μM)	EC_{90} (μM)	EC_{50} (μM)	EC_{90} (μM)
OBGS	$5.2 \times 10^{-2} \pm 1 \times 10^{-2}$	$7.5 \times 10^{-1} \pm 3 \times 10^{-2}$	$5.3 \times 10^{-4} \pm 8.1 \times 10^{-5}$	$5.3 \times 10^{-2} \pm 6.6 \times 10^{-3}$
DS	NI	NI	$5.4 \times 10^{-4} \pm 2.8 \times 10^{-5}$	$5.2 \times 10^{-2} \pm 7.6 \times 10^{-3}$

[a] Results show the mean ± SD from three to six replicates. [b] CD8-depleted peripheral blood mononuclear cells (PBMCs) were treated with serial 10-fold dilutions of native oat bran glucan (OBG), its sulfated derivative (OBGS), or dextran sulfate (DS) for 1 h and then washed free of the compounds prior to the addition of 100 tissue culture infectious doses ($TCID_{50}$) of HIV-1_{PE106}. NI is no inhibition. [c] 100 $TCID_{50}$ HIV-1_{PE106} was added to PBMCs and incubated for 2 h. Cells were washed free of unadsorbed virus prior to the addition of serial 10-fold dilutions of OBG, OBGS, or DS. EC_{50} is the concentration required to inhibit HIV-1 p24 antigen production by 50% and EC_{90} the concentration required to inhibit p24 production by 90% 7 days postinfection.

higher than the corresponding EC_{50}s shown in **Tables 3** and **4**. OBGS, but not DS, also inhibited syncytium formation in pretreated PBMCs at a concentration of 0.01 μM (not shown).

Cellular Cytotoxicity. Cytotoxicity was compared in untreated, uninfected PBMCs and mock-infected PBMCs incubated with serial log dilutions of the compounds (10 to 0.0001 μM) for 7 days using the Guava PCA-96 cell analyzer. Due to the high viscosity of OBG, the first dilution tested was 1 μM when the number of viable cells/mL was 81 ± 9.5% control PBMCs (not shown) so the CC_{50} could not be calculated. The CC_{50} for OBGS and DS were >9 and 7.67 μM, respectively (**Table 3**), and the SI derived from the CC_{50}/EC_{50} was higher for OBGS (>15050) than DS (9232)

DISCUSSION

In China, the annual yield of oats is approximately 465,000 tons (*3*). Oat bran is not suitable for animal feed nor valued for human consumption, and most is discarded yet is a valuable source of β-glucans that have wide health and medicinal value (*4–6, 24*). Thus to increase the economic and agricultural value of oat bran, we extracted the native glucan, OBG, and synthesized a sulfated derivative, OBGS. We used naked oats (*A. sativa* L.) as a source of bran rather than hulled oats, because the bran threshes free of the kernel more easily making the process more economical.

Structural analysis showed OBG to be a linear (1–3)-linked β-glucan with a (1–4)-linked backbone (**Table 2**) Analysis of the sulfated derivative, OBGS, showed the presence of 2,4-dimethylglucosyl and 2,3-dimethylglucosyl residues in ratios of 1:3. In comparison with OBG, the substituted position of the sulfate group was deduced to be position O-6 of glucose residues (**Table 2**). The sulfate content was 36.5%, which is more than 3 times that reported by Chang and others (*7*) who derived a sulfated β-glucan from oat endosperm. These workers reported the molecular weight of the oat endosperm β-glucan and its sulfated derivative to be 130 and 68 KDa, respectively. They used chlorosulfonic acid to directly sulfate the glucan, which resulted in degradation. The molecular weight of our oat bran β-glucan and sulfated derivatives were 500 and 686 KDa, respectively (**Table 1**), and under the sulfation conditions we used, chlorosulfonic−pyridine complex (*20*), no degradation of the native glucan occurred, and a higher substitutive derivative was achieved. Sulfation of OBG was also confirmed by FT-IR; the appearance of absorption bands at 1269 cm^{-1} in the OBGS IR spectra resulted from the vibration of S═O groups, and the β configuration was confirmed by the absorption at 890 cm^{-1} (**Figure 1**). Thus our β-glucan and its sulfated derivative differ from those reported by Chang and co-workers (*7*) who did not report the position of the substituted sulfate group nor study anti-HIV activity.

We compared the anti-HIV-1 properties of the OBG and OBGS with the antiviral compound DS (*13*), and to the best of our knowledge, this is the first such report for a sulfated oat bran derivative that inhibits HIV-1. Anti-HIV-1 activity was evaluated in a model based on a primary isolate of HIV-1 (PE106) and PBMCs rather than a prototype T-cell line adapted virus in continuous cell lines. Primary isolates are generally less sensitive to inhibition by polyanions (*26, 27*), whereas T-cell line adapted HIV-1 strains acquire a more basic V3 loop and become more susceptible to these compounds (*25, 26, 28*). Thus our model is more representative of the in vivo situation.

In general, soluble sulfated polysaccharides inhibit the first stage of infection in which HIV-1 binds to the cell surface via interaction between heparin sulfate proteoglycans (*13, 14*) and the V3 loop of the virus surface glycoprotein (gp120). Negatively charged sulfate groups bind to positively charged amino acid side chains, effectively shielding V3, preventing binding and fusion (*24*). The involvement of other regions of HIV-1 gp120 have also been demonstrated, including the CD4 and co-receptor binding sites (*25*). Thus antiviral activity is largely dependent on two factors, high molecular weight and high negative charge (*14*), and OBGS gave potent anti-HIV-1 activity pre- and postinfection (**Tables 3** and **4**) as reported for DS and other sulfated polysaccharides (*13, 29*). However, unlike DS, OBGS can also protect pretreated PBMCs from the higher virus challenge (**Table 4**).

Although low, OBGS cytotxicity was higher than that reported for some other sulfated polysaccharides (**Table 3**). However, cell death was examined by fluorescent dye exclusion

(10,000 cells/well) using the Guava PCA. This method differentiates between viable cells, cells at early stages of apoptosis, and dead cells. Early apoptotic cells are not detected in standard cytotoxicity assays such as MTT or XTT because they are metabolically active. In addition, PBMCs are more sensitive to cytotoxic effects than continuous cell lines (*26*). Nevertheless, OBGS is less cytotoxic than DS (**Table 3**) and exerts potent activity versus a high virus challenge (100 $TCID_{50}$ of PE106) at a concentration approximately 15,000 times below its cytotoxic concentration.

According to the 2007 UNAIDS global update, among the infectious diseases HIV-1 still presents the most serious public health challenge. There are more than 6,800 new infections and more than 5,700 HIV-1 associated deaths each day, most of which could be avoided if access to HIV-1 prevention and treatment services were available. Globally, of the 30.8 million HIV-1-infected adults, 15.4 million are women but in many low-income countries the ratio of HIV-1-infected women is higher. For example, 61% of adult infections are in women in sub-Saharan Africa where there is limited access to antiretroviral drugs, and as a result of socially constructed gender inequalities many women do not have the choice of using condoms. Thus increased research into the development of female-controlled methods of preventing HIV-1 transmission is required. We are particularly interested in the potential of sulfated glucans such as OBGS as virucidal vaginal gel preparations that would reduce HIV-1 transmission by preventing virus cell entry. In addition to potent inhibition of HIV-1, sulfated polysaccharides possess other properties including gel formation, modulation of CD4 expression (*24*), inhibition of cell-to-cell adhesions (*12–14*), low cellular cytotoxicity, and low selection for virus resistance in vitro (*14, 15*) that make them suitable for use in vaginal formulations. Several such candidate compounds are in current clinical trials (*30*).

We have shown that the β-glucan OBG extracted from what is essentially a waste product in China can be used to readily synthesize a derivative of high sulfate content that has low cytotoxicity in primary human cells. Moreover, the raw material can be converted to OBGS at a low cost of $20/g at laboratory-scale production. In addition to inhibiting HIV-1-infection OBGS is active postinfection and, of equal importance, protects pretreated PBMCs that have been washed free of the compound from infection. Thus OBGS possesses several of the properties required of a candidate vaginal viricide.

ABBREVIATIONS USED

PBMCs, peripheral blood mononuclear cells; HIV-1, human immunodeficiency virus; dpi, days postinfection; $TCID_{50}$, tissue culture infective dose 50; EC_{50}, the dilution required to inhibit p24 production by 50%; EC_{90}, the dilution required to inhibit p24 production by 90%; CC_{50}, the dilution required to kill 50% uninfected PBMCs; SI, selectivity index.

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