Short Paper

Inhibitory Effect of Sulfated Derivatives of a Marine Bacterial Polysaccharide on Replication of Human Immunodeficiency Virus *in Vitro*

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Although a inhibitory effect of sulfated polysaccharides such as dextran sulfate on the human immunodeficiency virus type 1 (HIV-1) has been reported,¹⁻³⁾ clinical trials have been unsuccessful in the therapy of acquired immune deficiency syndrome (AIDS) in humans because of their blood anticoagulant activity and toxicity caused by their high sulfate contents.

Sulfated derivatives prepared by a fucosamine-containing polysaccharide from a marine *Pseudomonas* sp. strain HA-318 showed strong antiviral activity against herpes simplex virus type 1 (HSV-1)⁴⁾ *in vitro*, even in their low sulfate contents. This paper describes the anti-HIV-1 effect of these sulfated polysaccharides *in vitro*.

The procedure for the isolation of the polysaccharide from bacterial culture, the preparation of the sulfated derivatives, and measuring the component sugars has been described previously.⁵⁾ Briefly, fucosamine-containing polysaccharide (1g) extracellularly produced by a marine *Pseudomonas* strain HA-318 was treated with chlorosulfonic acid (15 ml) in formamide (100 ml) in an ice-water bath for 15 min, and then incubated at 25°C for 1 h. After neutralization with solid Na₂CO₃, the reaction mixture was dialyzed against deionized water and then freeze-dried (1.4 g). The product (504 mg) was fractionated by chromatography on a DEAE-cellulose column (3.5×33.0 cm) using stepwise elution with 0.01 M phosphate buffer (pH 7.0) containing 0–2 M NaCl. Each fraction eluted with 0.6, 0.8, 1.0, and 2.0 M NaCl buffers was dialyzed against deionized water, followed by freeze-drying. Each fraction (A1, A2, A3, and A4) was found to be homogeneous electrophoretically. The products were composed in equal parts of galacturonic acid, galactose, N-acetylfucosamine, and pyruvate at a molar ratio of 3:1:1:1, together with sulfates. There were no significant differences in molecular size between them (Table 1).

Inhibitory activities of sulfated polysaccharides were evaluated against HIV-1 (HTLV-III_B strain) which was obtained from the culture supernatant of MOLT-4 cells persistently infected with the virus (MOLT-4/HTLV-III_B). The titer of the virus stock was 2×10⁵ CCID₅₀/ml (50% cell culture infective

dose per ml) and the virus was stocked at –80°C until use.

MT-4 cells were used for the anti-HIV-1 assay. The cell lines were grown and maintained in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 100 units/ml penicillin G, and 20 µg/ml gentamicin.

The activity of the compound against the replication of HIV-1 was based on the inhibition of virus-induced cytopathogenicity in MT-4 cells which were suspended in culture medium at 10⁵ cells/ml and infected with cell free HIV-1 at 1,000 CCID₅₀/ml. Immediately after virus infection, the cell suspension (100 µl) was brought into each well of a flat-bottomed microtiter tray containing 100 µl of various concentrations of the test compounds. After a 4-day incubation at 37°C, the number of viable cells was determined by the MTT method⁶⁾ based on the mitochondrial reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide. The cytotoxicity of the compounds was evaluated in parallel with their antiviral activity which was based on the viability of mock-infected cells, as monitored by the MTT method.

As shown in Table 1, the test samples of different sulfate contents showed different activities against HIV-1. The anti-HIV-1 activity of Fraction A1 (IC₅₀=0.69 µg/ml) was higher than that of dextran sulfate (IC₅₀=1.5 µg/ml). No cytotoxicity of these test samples for MT-4 cells was noted at concentrations up to 100 µg/ml. It should be noted that Fraction A1 showed an anti-HIV-1 effect at a lower degree of sulfation (S=5.6%, W/W) than dextran sulfate (S>17%, W/W)¹⁾ and heparin (S>10%, W/W).^{2,3)} In contrast, the native polysaccharide itself (before sulfation) did not show any anti-HIV-1 activity even at 100 µg/ml.

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References

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Table 1. Properties and anti-HIV-1 activity of the sulfated polysaccharide

Sample	Yields (mg)	S (% W/W)	M.W.* ¹	Anti-HIV-1	
				IC ₅₀ * ²	CC ₅₀ * ³
A1	327	5.6	1.9×10 ⁵	0.69	>100
A2	73	8.8	1.2×10 ⁵	1.5	>100
A3	26	9.9	2.3×10 ⁵	1.6	>100
A4	23	5.8	2.9×10 ⁵	2.2	>100
DS 500* ⁴				1.5	>100

*¹ Molecular weight was estimated by GPC on a Asahipak GFA 7M column with pullulan (Shodex standard kit P-82) as the standard.

*² Concentrations (µg/ml) required to reduce HIV-1 induced cytopathogenicity by 50%.

*³ Concentrations (µg/ml) required to reduce viable cells in a mock-infected culture by 50%.

*⁴ Dextran sulfate 5000 obtained from Sigma Chemical Co.

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