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Sulfated Polysaccharides Are Potent and Selective Inhibitors of Various Enveloped Viruses, Including Herpes Simplex Virus, Cytomegalovirus, Vesicular Stomatitis Virus, and Human Immunodeficiency Virus

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Several sulfated polysaccharides (dextran sulfate, pentosan polysulfate, fucoidan, and carrageenans) proved to be potent inhibitors for herpes simplex virus, human cytomegalovirus, vesicular stomatitis virus, Sindbis virus, and human immunodeficiency virus. They were moderately inhibitory to vaccinia virus but not inhibitory to adenovirus, coxsackievirus, poliovirus, parainfluenza virus, and reovirus. These results indicate that, with the exception of parainfluenza virus, enveloped viruses are specifically susceptible to the inhibitory activity of sulfated polysaccharides.

The inhibitory effects of polyanionic substances on the replication of herpes simplex virus (HSV) and other viruses were reported more than two decades ago (7, 11). However, these observations did not generate much interest because the antiviral action of the compounds was considered to be largely nonspecific. Interest on the antiviral potential of sulfated polysaccharides regained momentum when recent studies showed that dextran sulfate and heparin are highly inhibitory to the replication of human immunodeficiency virus type 1 (HIV-1) in vitro (9, 14). We have now evaluated several sulfated polysaccharides for their activities against a variety of DNA ar.d RNA viruses and found that, as a rule, these compounds are potent and selective inhibitors of enveloped viruses, irrespective of whether these were DNA or RNA viruses.

Dextran sulfates (approximate molecular weights [MW] of 1,000, 10,000, 40,000, 70,000, 110,000, and 500,000) were obtained from Pfeifer & Langen, Dormagen, Federal Republic of Germany. Heparin was provided by W. O. Godtfredsen, Leo Pharmaceutical Products Ltd., Ballerup, Denmark. Dextran sulfate (MW, 5,000), pentosan polysulfate, fucoidan, and κ- and λ-carrageenan were purchased from Sigma Chemical Co., St. Louis, Mo. The viruses were obtained as follows: HSV type 1 (HSV-1) (KOS strain), HSV-2 (G strain), and the thymidine kinase-deficient (TK^-) mutant of HSV-1 (B 2006 strain), from De Clercq et al. (3); human cytomegalovirus (CMV) (Davis strain), from De Clercq et al. (4); adenovirus type 2, from Baba et al. (1); vaccinia virus, vesicular stomatitis virus (VSV), coxsackievirus type B4, poliovirus type 1, and Sindbis virus, from De Clercq et al. (5); and HIV-1 (human T-cell lymphotropic virus type III_B [HTLV-III_B] strain) from Pauwels et al. (12); parainfluenza virus type 3 (ATCC VR-93) and reovirus type 1 (ATCC VR-230) were obtained from the American Type Culture Collection (Rockville, Md.). HIV-1 was obtained from the culture supernatant of a persistently HIV-1-infected HUT-78 cell (HUT-78/HTLV-III_B). The HTLV-I-carrying T4 cell line, MT-4, is described elsewhere (8, 12). The Vero, HeLa, and MT-4 cell lines used in this study were regularly examined for mycoplasma contamination and found to be mycoplasma free.

Except for the procedure of the anti-HIV-1 assay, the procedures for assays have been described previously (1–5). Confluent cell cultures in microdilution trays were exposed to 100 50% cell culture infective doses of virus per well in the presence of various concentrations of the test compounds. After 1 h of adsorption at 37°C, the residual virus was replaced by culture medium (Eagle minimum essential medium supplemented with 3% fetal calf serum and antibiotics) containing the test compounds in the same concentrations used during the virus adsorption period. Virus-induced cytopathogenicity was recorded at 1 to 2 days for VSV; at 2 days for coxsackievirus and poliovirus; at 2 to 3 days for HSV-1, HSV-2, TK^- HSV-1, vaccinia virus, and Sindbis virus; and at 6 to 7 days for CMV, adenovirus, parainfluenza virus, and reovirus. The antiviral activity of the compounds is expressed as the MIC or the concentration required to inhibit virus-induced cytopathogenicity by 50%. For CMV, antiviral activity was expressed as the concentration required to reduce the number of plaques by 50% (6).

The activity of the compounds against HIV-1 was monitored by the inhibition of HIV-1-induced cytopathogenicity in MT-4 cells. Briefly, MT-4 cells (3×10^4 cells per well) were cultured in microdilution trays in the presence of various concentrations of test compounds added immediately after infection with 100 50% cell culture infective doses of HIV-1. After 5 days of incubation at 37°C, the number of viable cells was determined by the MTT (3′-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) method (11a).

The cytotoxicity of the compounds was assessed by several parameters, as previously described (2, 6): (i) alteration of normal cell morphology, (ii) inhibition of cell proliferation, and (iii) inhibition of host cell macromolecule (DNA and RNA) synthesis on the basis of the incorporation of [*methyl*-^{3}H]thymidine and [5-^{3}H]uridine, respectively.

When six sulfated polysaccharides, i.e., dextran sulfate (MW, 10,000), heparin, pentosan polysulfate, fucoidan, and κ- and λ-carrageenan, were examined for their inhibitory effects on the replication of DNA viruses, all compounds were inhibitory to herpesviruses (HSV-1, HSV-2, TK^-

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TABLE 1. Inhibitory effect of sulfated polysaccharides on replication of DNA and RNA viruses

Virus	Cell culture	MIC[a] (μg/ml)					
		Dextran sulfate (MW, 10,000)	Heparin	Pentosan polysulfate	Fucoidan	κ-Carrageenan	λ-Carrageenan
HSV-1	PRK	2	37	3.7	1.7	3.7	1.6
HSV-2	PRK	0.5	32	5.3	1.1	2	1.5
TK⁻ HSV-1	PRK	1.3	20	4.5	1.5	15	4.5
CMV	HEL	0.5	21	1.7	2	2.8	0.3
Adenovirus type 2	HEL	>200	>200	>200	>200	>200	>200
Vaccinia virus	PRK	16	≥200	10	≥200	36	16
VSV	PRK	0.3	7	24	0.3	0.3	0.2
	HeLa	0.5	300	8	11	7	4
Coxsackievirus type B4	HeLa	>400	>400	>400	>400	>400	>400
Poliovirus type 1	HeLa	>400	>400	>400	>400	>400	>400
Parainfluenza virus type 3	Vero	>400	>400	>400	>400	>40[b]	>4[b]
Reovirus type 1	Vero	>400	>400	70	>400	>40[b]	>4[b]
Sindbis virus	Vero	3.5	200	20	7	7	2
HIV-1	MT-4	0.5	1.9	0.7	2.8	12	1.9

[a] Concentration required to inhibit virus-induced cytopathogenicity by 50%; mean values for two or three experiments.
[b] Cell morphology was altered at these or higher concentrations.

HSV-1, and CMV) (Table 1). Of the six compounds tested, dextran sulfate (MW, 10,000) and fucoidan were the most potent inhibitors of herpesviruses, and heparin was the weakest. Vaccinia virus was inhibited only at relatively high concentrations of the compounds (10 to 200 μg/ml). Adenovirus replication was not affected by any of the compounds, even at 200 μg/ml (Table 1).

When the six sulfated polysaccharides were evaluated for activity against RNA viruses, the compounds were found to be active against VSV, Sindbis virus, and HIV-1. However, the compounds were inactive against coxsackievirus, poliovirus, and parainfluenza virus (Table 1). Similarly, reovirus was not susceptible to the inhibitory effects of the compounds except for pentosan polysulfate (MIC, 70 μg/ml). HIV-1 was very susceptible to all sulfated polysaccharides; VSV also showed high susceptibility (Table 1).

Except for κ- and λ-carrageenan, which affected Vero cells, none of the test compounds disturbed normal cell morphology at a concentration of 200 μg/ml (for PRK and HEL cells) or 400 μg/ml (for HeLa and Vero cells) (data not shown). The morphology of Vero cells was altered by κ- and λ-carrageenan at concentrations of 40 and 4 μg/ml, respectively. The compounds were not inhibitory to the cell proliferations of HEL and MT-4 cells at concentrations ranging from 100 to 400 μg/ml. Neither DNA nor RNA synthesis of PRK cells was inhibited by any of the compounds at 100 μg/ml (data not shown). Thus, the sulfated polysaccharides were not toxic to the host cells at concentrations that were at least 100-fold in excess of those found to be inhibitory to virus replication.

A marked difference was found in the susceptibility to heparin of VSV in PRK and HeLa cells (Table 1). This finding suggested that there are possible differences in the virus-inhibitory effects of the sulfated polysaccharides, depending on the choice of cell line. Therefore, the inhibitory effect of the sulfated polysaccharides on VSV was further examined in a variety of cell lines. Except for heparin, all compounds showed marked antiviral activity in all cell lines tested (Table 2). However, the anti-VSV activity was most pronounced in PRK cells. This effect was shown not only by heparin but also by fucoidan and κ- and λ-carrageenan.

Various samples of dextran sulfate with MWs ranging from 1,000 to 500,000 was examined for their antiviral activity. A marked increased in antiviral activity, irrespective of the virus (vaccinia virus, HSV-1, TK⁻ HSV-1, HSV-2, CMV, Sindbis virus, HIV-1, and VSV), was observed when the MW of dextran sulfate increased from 1,000 to 10,000 (Fig. 1). When the MW further increased from 10,000 to 500,000, the antiviral activity of dextran sulfate tended to level off. Thus, a MW of 10,000 seems optimal for the virus-inhibitory effect of dextran sulfate.

It has been postulated that anionic polysaccharides can

TABLE 2. Inhibitory effect of sulfated polysaccharides on VSV in different cell lines

Cell culture	MIC[a] (μg/ml)					
	Dextran sulfate (MW, 10,000)	Heparin	Pentosan polysulfate	Fucoidan	κ-Carrageenan	λ-Carrageenan
PRK fibroblast[b]	0.3	7	24	0.3	0.3	0.2
HeLa[b]	0.5	300	8	11	7	4
Vero	1	200	20	2	7	2
HEL fibroblast	1	>400	20	2	7	2
Human embryonic skin-muscle (E_6SM) fibroblast	0.7	200	40	4	7	2
Rabbit kidney (RK13) fibroblast	2	>400	70	7	10	2
Monkey kidney (BSC-1A)	0.7	>400	7	2	7	2

[a] Concentration required to inhibit virus-induced cytopathogenicity by 50%.
[b] Data taken from Table 1.

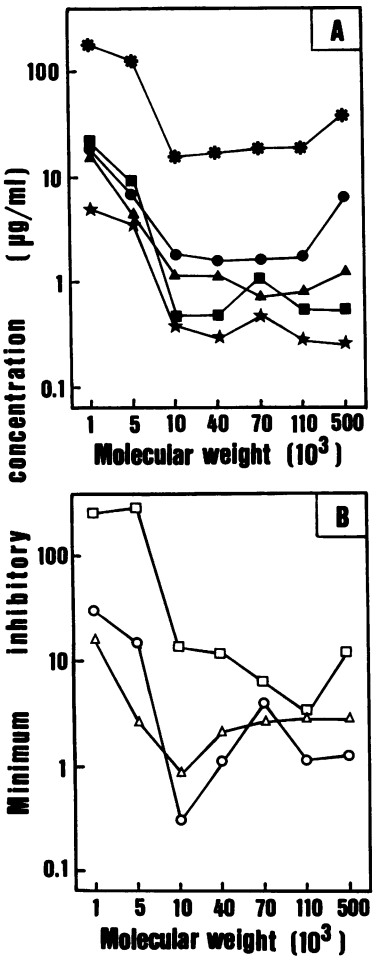

FIG. 1. Influence of molecular weight on activity of dextran sulfate against DNA (A) and RNA (B) viruses. Antiviral activity is expressed as MIC, the concentration required to inhibit virus-induced cytopathogenicity by 50% (mean values for two separate experiments). Symbols: *, vaccinia virus; ●, HSV-1; ▲, TK⁻ HSV-1; ■, HSV-2; ★, CMV; □, Sindbis virus; △, HIV-1; ○, VSV (in PRK cells).

interfere with a very early stage of the virus replication cycle, presumably virus adsorption (7). To assess the possible inhibitory effect of dextran sulfate (MW, 10,000) on the virus adsorption stage, two treatment regimens, A and B, were installed. In treatment A, the cells were exposed to the virus in the presence of the test compounds and incubated for 1 h at 37°C (normal treatment). In treatment B, the cells were exposed to the virus in the absence of the compound and incubated for 1 h at 37°C. After either treatment A or B, unadsorbed virus was removed, and the cells were further incubated in the presence of the compounds. The virus-inhibitory effect of dextran sulfate (MW, 10,000) was considerably reduced, if not completely eliminated, when the compound was added only after virus adsorption had taken place (Table 3).

The present study clearly indicates that sulfated polysaccharides have a broad-spectrum antiviral activity against enveloped viruses. In particular, dextran sulfate is a potent inhibitor of CMV and HIV-1 (Table 1). The MIC of dextran sulfate (MW, 10,000) for CMV and HIV-1 is 0.5 μg/ml, whereas the compound is not inhibitory to host cell (HEL or MT-4) proliferation even at 400 μg/ml (data not shown). This

TABLE 3. Differences in antiviral activity of dextran sulfate (MW, 10,000) added during or after virus adsorption

Virus	Cell culture	MIC[a] (μg/ml)	
		Treatment A	Treatment B
HSV-1	PRK	2	70
HSV-2	PRK	0.5	>400
Vaccinia virus	PRK	16	>400
VSV	PRK	0.3	400
HIV-1	MT-4	0.5	10

[a] Concentration required to inhibit virus-induced cytopathogenicity by 50%. Virus was inoculated in the presence (treatment A) or absence (treatment B) of the compound. After 60 min, unadsorbed virus was removed, and the cells were cultured in the presence of the compound (treatments A and B).

result means that, on the basis of the ratio of the MIC for host cell proliferation to the MIC for virus replication, the selectivity index of dextran sulfate (MW, 10,000) is more than 800. Thus, dextran sulfate and its congeners hold great promise for the treatment of CMV and HIV-1 infections, which is particularly advantageous for patients with acquired immunodeficiency syndrome who are prone to both infections and in whom the same compound may be used not only to suppress the causative agent (HIV-1) but also to prevent or suppress exacerbations of the opportunistic passenger (CMV) (13).

The mechanism of action of sulfated polysaccharides has been attributed to the inhibition of virus adsorption to the host cells (7). This phenomenon was not further addressed until it was recently proven that dextran sulfate and heparin block the attachment of HIV-1 particles to target T lymphocytes (1b, 10). The present observations (Table 3) suggest that dextran sulfate blocks the binding of HIV-1 and other enveloped viruses (i.e., HSV-1, HSV-2, vaccinia virus, and VSV) to cells. The activity of dextran sulfate against HSV-1 or -2 and VSV may form the basis for a bioassay to monitor levels of the compound in blood following its administration to patients.

Before sulfated polysaccharides can be recommended for treatment of virus infections, further investigations of their in vivo antiviral activity, pharmacokinetics, and toxicology are required. Heparin and sulfated polysaccharides have anticoagulant activity, and obviously, this activity may hamper their practical usefulness. However, we have previously demonstrated that dextran sulfate, pentosan polysulfate, fucoidan, and carrageenans achieve their in vitro inhibitory effect on HIV-1 at concentrations that are more than 100-fold lower than the anticoagulant threshold (1 IU) (1a). From the data presented in this paper, it appears that the compounds are about equally inhibitory to several RNA and DNA viruses other than HIV-1, including HSV-1, HSV-2, CMV, and VSV. Thus, dextran sulfate and its congeners are inhibitory to a variety of enveloped viruses at concentrations far below their anticoagulant threshold. This activity points to their potential as drugs for the chemotherapy of virus infections.

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