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Effect of polyionic compounds on the adsorption of polyoma virus

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Abstract

The inhibitory effect of polyionic compounds, including dextran sulfates and poly-L-lysines of various molecular weight, on the cytopathogenicity of polyoma virus was studied. Poly-L-lysines were found to be more potent inhibitors of polyoma virus than dextran sulfates in UC1B cells. The 50% effective concentrations (EC_{50}) were found to be inversely related to the molecular weight of the polymers. The higher the molecular weight of poly-L-lysine, the more effective was the polymer in inhibiting virus-induced cytopathogenicity. Incubation of the cells with poly-L-lysines was found to increase the zeta potential of the cells, whereas no significant change of the electrokinetic behavior was observed for dextran sulfate-treated cells, indicating that poly-L-lysines were adsorbed to the cells and prevented virus adsorption through steric hindrance effect. Studies using ^{3}H-labeled virions confirmed that poly-L-lysines inhibit virus adsorption to the host cells. The results obtained from this study indicated that poly-L-lysiness bind preferentially to UC1B cells through electrostatic interactions, whereas dextran sulfates are not effective inhibitors of polyoma virus.

Keywords: Dextran sulfates; Poly-L-lysines; Polyoma virus; Adsorption

1. Introduction

Several polycationic substances, such as poly-L-lysines, and polyanionic compounds, such as dextran sulfates and heparin, have been reported to be potent inhibitors of various viruses, including human immunodeficiency virus (HIV), herpes simplex virus (HSV), cytomegalovirus (CMV) and respiratory syncytial virus (RSV) (Schols et al., 1990; Witvrouw et al., 1991; Hosoya et al., 1991). The antiviral activities of these compounds are limited to enveloped viruses. The mechanism of antiviral action of these compounds was attributed to an inhibition of virus adsorption to the host cell membranes (Baba et al., 1988; Mitsuya et al., 1988; Langeland et al., 1988; Schols et al., 1990).

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Polyoma virus is a small DNA tumor virus, which transforms mouse and other rodent cells and can produce tumors in rodents. The initial step of infection of permissive cells by polyoma virus is the adsorption of the virions to the surface of host cells (Bolen and Consigli, 1979). Polyoma virus binds to the cell membranes through two putative physical mechanisms, (i) the hydrophobic interaction between the hydrophobic polyoma virus capsid proteins and the phospholipid hydrocarbon layer of the cell membranes and (ii) the electrostatic interaction between negatively charged membrane surface and the positively charged groups of the virus capsid proteins. Adsorption of polyoma virus capsids is followed by phagocytic engulfment and degradation in cell lysosomes (Mackay and Consigli, 1976). The present study was undertaken to evaluate the specificity of the inhibitory effect of polyionic compounds, including dextran sulfates and poly-L-lysines, on the cytopathogenicity of polyoma virus with regard to permissive cells such as UC1B, and the dependence of this effect on the molecular weight of the polymers.

To study the surface characteristics of the cells in the presence of the polymers, the microelectrophoretic technique was employed to determine the electrokinetic behavior of the cells. The surface charge measured reflects the state of the cell membrane and determines the local electric field adjacent to the cell surface, both of which play important roles in determining the interactions between cells and other biologically active substances in the vicinity of the membranes. The presence of dipoles and electric charges at the membrane surface also can enhance or weaken protein binding. It is speculated that adsorption of polyionic compounds to the cell surfaces would produce a change in the electrostatic potential at the surface of the membranes, which in turn would affect the virus–cell membrane interactions. The electrokinetic approach was employed here to determine the electrostatic potential of the cell membranes in the presence of polyionic substances in an attempt to address two questions: (a) how does the zeta potential of the cell membranes change in the presence of polyionic compounds? and (b) how does the physical state of cell membranes affect adsorption of polyoma virus? The answers to these questions are expected to give some hints as to the physical roles of polyionic substances on inhibition of viral cytopathicity. This paper will discuss from a physicochemical point of view the possible different effects caused by oppositely charged polyionic compounds on the adsorption of polyoma virus.

2. Materials and methods

2.1. Cells and virus

Mouse embryo cells, 3T6 (Todaro and Green, 1963) and UC1B (Hackett and Sylvester, 1972), were obtained from the American Type Culture Collection (ATCC) and maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 2% fungizone and 5% heat-inactivated calf serum.

Polyoma virus strain LID-1 (ATCC VR-252) was propagated in 3T6 cells. Usually cells were infected at a low multiplicity of infection (m.o.i.). Infectious viruses were plaque-titrated in UC1B cells and quantified as plaque-forming units (PFU) per ml. When extensive cytopathic effects were observed, the mixture of cells and medium was frozen and thawed three times, clarified by low-speed centrifugation, and stored at -20°C before use.

2.2. Compounds

Dextran sulfates (MW 8000, 50 000 and 500 000) and poly-L-lysines (MW 3000, 9000, 20 000, 100 000 and 300 000) were obtained from Sigma Chemical (St. Louis, MO).

2.3. Preparation of radiolabeled viruses

Following removal of the virus inoculum, 3T6 cells were overlaid with DMEM containing 10 μCi [^{3}H-*methyl*]thymidine (25 Ci/mmol) per ml of medium and incubated at 37°C. Virus was purified from the infected cell lysates following the procedures as described previously (Turler

and Beard, 1985). Fractions were collected with appropriate fractions pooled, and dialyzed against HEPES buffer (10 mM HEPES, 1 mM $CaCl_2$, 1 mM $MgCl_2$ and 5 mM KCl, pH 7.9).

2.4. Antiviral activity assay

Inhibition of virus-induced cytopathic effect was determined in a serial dilutions of compounds as described (De Clercq et al., 1980; De Clercq, 1985). Briefly, UC1B cells were maintained in 96-well tissue culture plates and infected with polyoma virus at 100 $TCID_{50}$ (50% tissue culture infective dose) per well in the presence of various concentrations of polymers. The cell cultures were further incubated at 37°C until virus-induced cytopathicity was completed in the virus-infected, polymer-free control cell cultures. The concentration required to reduce virus-induced cytopathogenicity by 50% was estimated as the 50% effective concentration (EC_{50}) (Hosoya et al., 1991).

2.5. Cytotoxicity assay

The concentration required to reduce the viability of cells by 50% was estimated as the 50% cytotoxic concentration (CC_{50}) (Hosoya et al., 1991). The number of viable cells was determined microscopically in a hemacytometer by the trypan blue exclusion method.

2.6. Microelectrophoresis measurements

UC1B cells were incubated in DMEM containing 50–450 μg/ml dextran sulfate or poly-L-lysine at 4°C for 6 h. Cells were then washed with phosphate buffered saline (PBS), trypsinized off the petri dishes and centrifuged at 1000 rpm for 3 min. The cell pellet was resuspended in the microelectrophoresis medium containing 10 mM glycylglycine, 2.5 mM KCl and 280 mM sucrose, buffered to various pH.

The electrophoretic mobilities of UC1B cells were measured in a flat-rectangular cell at 37°C with a Rank Brothers Mark II microelectrophoresis apparatus (Cambridge, United Kingdom). The binocular microscope head was fitted to a Philip video camera LDH 402 linked to a television monitor. About 10–15 cells were observed at each stationary level with polarity of the platinum electrodes being reversed between successive observations to minimize polarization effects. The zeta-potential values, ζ, of the cells (ζ = the electrical potential measured at the plane of shear, considered to lie close to the surface where the fluid is stationary) were calculated from the electrophoretic mobility, u, using the Helmholtz-Smoluchowski equation (Hunter, 1981):

$$\zeta = \frac{4\pi\eta \mathrm{u}}{\varepsilon},$$

where η is the viscosity, ε is the dielectric constant of the medium, and u is the electrophoretic mobility, calculated by dividing the electrophoretic velocities by the strength of the electric field.

2.7. Virus adsorption assay

UC1B cells were maintained at 37°C in 6-well cluster plates containing various concentrations of the test compounds. About 3×10^6 PFU of ^{3}H-labeled polyoma virus was added to each well. The cells were incubated at 4°C for 4 h allowing for virus adsorption. The virus inoculum was then removed, and the cells were rinsed thoroughly with PBS to remove the adsorbed virus particles and then lysed. The cell lysates were dissolved in Ecosint (National Diagnostic, England), and the radioactivity was counted by a Beckman LS6000IC liquid scintillation counter.

3. Results

3.1. Anti-polyoma virus activity of dextran sulfate and poly-L-lysines

Dextran sulfates and poly-L-lysines were examined for their inhibitory effects on the cytopathicity of polyoma virus in UC1B cells. Experimental data shown in Table 1 demonstrated that poly-L-lysines exhibited a better anti-polyoma virus activity than dextran sulfates. The EC_{50} values of poly-L-lysine range from 8 μg/ml (MW 300 000) to 110 μg/ml (MW 3000), showing that the higher

the molecular weight of the polymer, the more effective are the antiviral activities. The CC_{50} values of poly-L-lysines were also found to decrease with increasing molecular weight, indicating that the cytotoxicity of poly-L-lysines increased with increasing molecular weight. A similar molecular-weight dependence of EC_{50} values was also observed in the dextran sulfate-treated cells. However, the relatively high EC_{50} values of dextran sulfates suggested that they are not effective inhibitors of the polyoma virus in vitro.

3.2. Electrokinetic measurements

In order to examine the effect of polymer adsorption on the electrokinetic behavior of UC1B cells, cells were incubated with polyionic compounds at 4°C for 6 h, washed with PBS, trypsinized, and the electrophoretic mobilities were measured in the stationary layer. The zeta-potential value of UC1B cells was shown to vary with the pH of the microelectrophoresis medium (Fig. 1). Their value was approximately -53 mV at pH 7.4, with the isoelectric point of the cells occurring at pH 4.3. Figs. 2 and 3 show the effect of the concentration of the polyionic compounds on the zeta potential of UC1B cells. Incubation of cells with dextran sulfates (MW 8000, 50 000 and 500 000) up to 450 μg/ml does not significantly change the zeta potential of the cells (Fig. 2), indicating that dextran sulfates do not adsorb onto the cells. Addition of poly-L-lysines, on the other hand, was shown to increase the zeta potential of the cells, which increases from approximately -55– -53 mV in the absence of poly-L-lysines to $+14$– $+27$ mV in the presence of 450 μg/ml poly-L-lysines. The changes of surface potential of the cells were found to depend on the molecular weight and concentration of the polymers in the medium. The higher the concen-

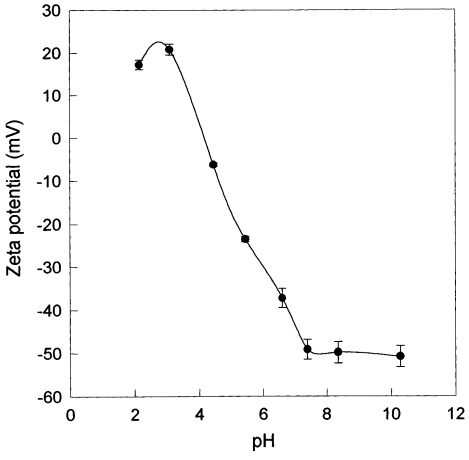

Fig. 1. Zeta potentials of UC1B cells as a function of pH. Data represent the averages of 10–15 cells, with S.D. indicated.

Table 1
Anti-polyoma virus activity and cytotoxicity of dextran sulfates and poly-L-lysines in UC1B cells

Compound	MW	EC_{50} (μg/ml)[a]	CC_{50} (μg/ml)[b]
Poly-L-lysine	3000	110	350
Poly-L-lysine	9000	32	150
Poly-L-lysine	20 000	20	50
Poly-L-lysine	100 000	12	40
Poly-L-lysine	300 000	8	35
Dextran sulfate	8000	350	>450
Dextran sulfate	50 000	250	>450
Dextran sulfate	500 000	216	>450

[a] 50% Effective concentration, or concentration required to reduce virus-induced cytopathogenicity by 50%.
[b] 50% Cytotoxic concentration, or concentration required to reduce the cell viability by 50%.

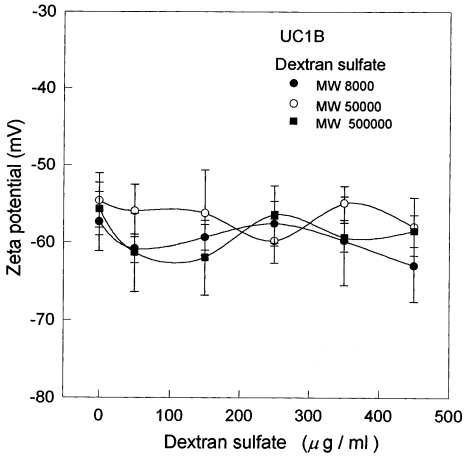

Fig. 2. Zeta potentials of UC1B cells (pH 7.4) in DMEM containing various concentrations of dextran sulfates. Molecular weights of dextran sulfates are: (●), MW 8000; (○), MW 50 000; ■, MW 500 000. Data represent the average measurements of 10–15 cells, with S.D. indicated.

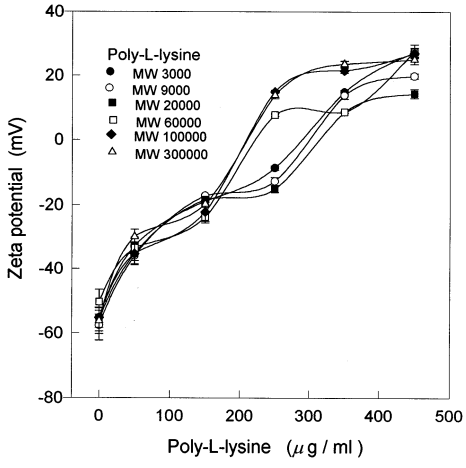

Fig. 3. Zeta potentials of UC1B cells (pH 7.4) in DMEM containing various concentrations of poly-L-lysines. Molecular weights of poly-L-lysines are: (●), MW 3000; (○), MW 9000; ■, MW 20 000; □, MW 60 000; ◆, MW 100 000; △, MW 300 000. Data represent the average measurements of 10–15 cells, with S.D. indicated.

tration of poly-L-lysine, the greater the change in the zeta potential observed (Fig. 3). Charge reversal of the cells was shown to occur at poly-L-lysine concentrations between 200 and 300 μg/ml, suggesting that UC1B cells become more positively charged when incubated with poly-L-lysine at concentrations above this range.

3.3. Inhibitory effect of polymers on polyoma virus adsorption

To determine whether the polyanionic and polycationic compounds are inhibitory to polyoma virus adsorption to the UC1B cells, [^{3}H-*methyl*]thymidine-labeled polyoma virus was allowed to adsorb to the cells at 4°C for 4 h in the presence of the test compounds. Remaining unadsorbed virus was subsequently removed. The cells were thoroughly rinsed and lysed. The radioactivity was then counted by a scintillation counter. Figs. 4 and 5 show that both dextran sulfates and poly-L-lysines inhibited the adsorption of polyoma virus. The inhibitory effect is dependent upon the concentration and molecular weight of the polymers. In general, the higher the molecular weight and concentrations, the more effective are the polymers in inhibiting the virus adsorption onto the cells. However, dextran sulfate of MW 8000 showed an almost negligible effect on polyoma virus binding to the cells. Maximum inhibition of 41% and 54% was obtained for dextran sulfates (450 μg/ml) of MW 50 000 and 500 000, respectively. Poly-L-lysines, on the other hand, were found to exert greater inhibitory effect on the adsorption of polyoma virus onto UC1B cells. Greater than 90% inhibition was achieved for poly-L-lysine of MW 300 000 at a concentration of 450 μg/ml, which was considerably greater than for dextran sulfates at the equivalent concentrations.

4. Discussion

Polyanionic and polycationic substances, including dextran sulfates and poly-L-lysines, were evaluated in this study for their inhibitory effects on the cytopathogenicity of polyoma virus. The relatively high EC_{50} values of dextran sulfates,

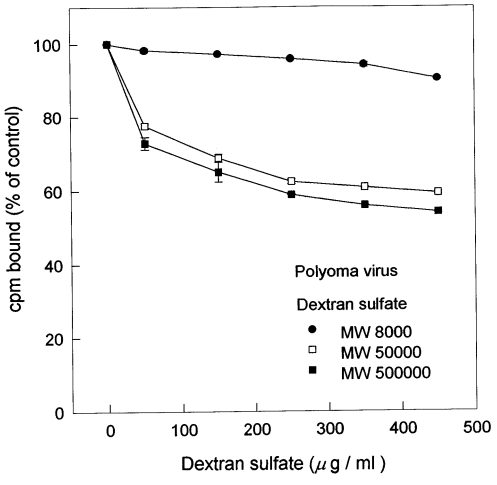

Fig. 4. Effect of dextran sulfates on the adsorption of ^{3}H-labeled polyoma virus to UC1B cells. The cells were incubated with dextran sulfate of MW 8000 (●), 50 000 (○) and 500 000 (■), respectively, and ^{3}H-labeled HSV-1 was then added. The samples were incubated at 4°C for 4 h. At the end of incubation, the cells were washed with PBS to remove unadsorbed virus particles, trypsinized and then lysed. The radioactivity was counted in a β-scintillation counter. Data are presented as mean values of triplicate sets of experiments, with S.D. indicated.

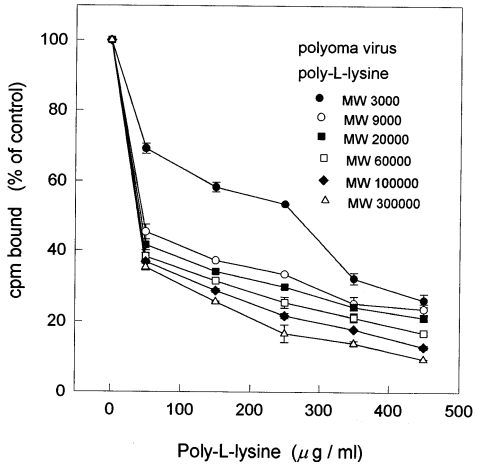

Fig. 5. Effect of poly-L-lysines on the adsorption of ^{3}H-labeled polyoma virus to UC1B cells. The cells were incubated with poly-L-lysine of MW 3000 (●), 9000 (○), 20 000 (■), 60 000 (□), 100 000 (◆) and 300 000 (△), respectively, and ^{3}H-labeled HSV-1 was then added. The samples were incubated at 4°C for 4 h. At the end of incubation, the cells were washed with PBS to remove unadsorbed virus particles, trypsinized and then lysed. The radioactivity was counted in a β-scintillation counter. Data are presented as mean values of triplicate sets of experiments, with S.D. indicated.

ranging from 216 μg/ml (MW 500 000) to 350 μg/ml (MW 8000), suggest that these polyanionic compounds are not effective inhibitors of polyoma virus. Poly-L-lysines, on the other hand, showed EC_{50} values ranging from 8 μg/ml (MW 300 000) to 110 μg/ml (MW 3000). These values were all lower than the CC_{50} values of the corresponding poly-L-lysines (Table 1). The results obtained indicated that poly-L-lysines are potent inhibitors of polyoma virus in vitro and that the inhibitory effect is dependent on the molecular weight of the polymers.

In order to examine the interactions of the ionic polymers with UC1B cells, cells in the presence of the polymers were examined microelectrophoretically as a function of concentration of the polymers. Results presented in Figs. 1–3 show that UC1B cells are negatively charged at the physiological pH of 7.4. Addition of dextran sulfates does not significantly change the zeta-potential values of the cells, whereas incubation of cells with poly-L-lysines renders the cells more positively charged. The results suggest that dextran sulfates do not adsorb onto the cell surfaces as do poly-L-lysines. The extent of adsorption was dependent on the concentration and molecular weight of the polymers. The higher the molecular weights of poly-L-lysines, the more effective were the polymers in changing the membrane surface charges of the cells. Most biological membranes carry negative charges. The binding mechanism of these polymers to the cells is therefore electrostatic in nature, such that the positively charged poly-L-lysines can bind with negatively charged groups on the cell membrane surfaces, while negatively charged dextran sulfates cannot. The data on the cytopathogenicity of the virus suggest that receptor binding was probably inhibited in the presence of polymers (Table 1). Interaction of poly-L-lysines with the cell membrane surfaces thus inhibits adsorption of the virus.

The relatively low inhibitory effect of dextran sulfates on polyoma virus adsorption, compared with poly-L-lysines, was also evidenced in Figs. 4 and 5, where the maximum inhibitory effect of dextran sulfates on binding of ^{3}H-labeled virus onto UC1B cells was 54% (MW 500 000) at a concentration of 450 μg/ml. In contrast, poly-L-lysine (MW 300 000) at the same concentration can achieve a maximum inhibition of ^{3}H-labeled virus adsorption of up to 91%, suggesting that the small inhibitory effect of dextran sulfates on the cytopathogenicity of polyoma virus, as shown in Table 1, is partly attributed to this marginal effect on virus adsorption by the polymers, the reasons for which are unclear at this point. Poly-L-lysines, on the other hand, inhibit virus binding by being adsorbed onto the cell surfaces, mainly through electrostatic forces. The oppositely charged interactions between polymers and cells not only reduce the hydrophobic and electrostatic binding between virions and UC1B cells, but also suppress the virus attachment due to steric interference. The inhibitory effects of these polymers were dependent on concentration and molecular weight, indicating that polymers of higher molecular weight are more effective in inhibiting virus adsorption, presumably due to higher degrees of entanglements and couplings of the polymer chains. The molecular-weight dependence of inhibitory effect was paralleled in the results for

virus-induced cytopathicity (Table 1). This shielding effect appeared to become more pronounced as concentration of polymers increased.

The results obtained in this study demonstrated that poly-L-lysines with sufficient molecular weight are effective in inhibiting polyoma virus infection. The inhibition mechanism is ascribed to the adsorption of polymers onto the host cells through electrostatic interactions which prevent the attachment of virus particles to the cell surfaces by the steric hindrance effect. Dextran sulfates, on the other hand, are not effective inhibitors of polyoma virus.

Acknowledgements

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