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# Effect of anionic polymers on fusion of Sendai virus with human erythrocyte ghosts

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**Summary**

The effect of anionic polymers (dextran sulfate, heparin and chondroitin sulfate) on fusion of Sendai virus with erythrocyte ghosts was studied. The effect of pH on the activity of these anionic polymers was also investigated. In order to examine the interaction of such polymers with the Sendai virion and erythrocyte ghost surfaces, the binding of virions to erythrocyte ghosts and the aggregation of virions and/or erythrocyte ghosts were also measured with respect to the same parameters. It was found that the anionic polymers suppressed the fusion of Sendai virus with erythrocyte ghosts. The order of effectiveness of the polymers in suppression was dextran sulfate > heparin > chondroitin sulfate, for the application of a same quantity (weight/ml) of the polymers. The lower the pH of the suspending medium, the more effective were the polymers in suppressing virion-erythrocyte ghost aggregation and fusion. The suppression of fusion was dependent on the concentration of the polymers applied: the higher the concentration of the polymer applied, the more the suppression was observed. Evidence from binding studies, turbidity measurements and electrophoretic mobility measurements indicates that the anionic polymers interact preferentially with the virion surface.

Anionic polymer; Sendai virus; Human erythrocyte ghost

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## Introduction

The fusion of Sendai virus with erythrocyte ghosts has been studied by a number of workers (Howe and Morgan, 1969; Maeda et al., 1975; Lalazar and Loyter, 1979; Sekiguchi and Asano, 1978; Wolf et al., 1980; Ozawa et al., 1979; Sekiguchi et al., 1981; Hoekstra et al., 1984; Hoekstra and Klappe, 1986). The two major virus envelope proteins, hemagglutinin-neuraminidase (HN) and fusion (F) have been postulated to be involved in the fusion mechanism for this membrane system (Poste and Pasternack, 1978; Hsu et al., 1981; Merz et al., 1981; Loyter and Volsky, 1982; Hoekstra and Klappe, 1986; Ohnishi, 1988). The former protein (HN) is considered to bind to specific glycoproteins and glycolipids of the host cells, especially sialoglycoproteins or gangliosides, to bring the virion and the host cell close together. The negatively charged sites of the host glycoproteins may be responsible for binding with the HN protein. The F protein consists of two polypeptide chains ($F_1$ and $F_2$). The amino terminal of F has a highly hydrophobic peptide portion, which presumably interacts with the host cell and may penetrate into the host cell membrane (Novick and Hoekstra, 1988), thus inducing fusion of the two interacting membranes. However, some recent studies indicate that the HN protein also plays some role in membrane fusion (Henis et al., 1989).

Glycosamino glycans (GAG) comprised of negatively charged macromolecules such as heparin and chondroitin sulfate, are important components of the extra cellular matrix. Dextran sulfate has a similar structure and has been used as a model for studies on GAG. Dextran sulfate has an ability to inhibit clotting of blood and has been used as an anticoagulant and as a plasma expander (Facts and Comparisons, 1986). Some studies have also shown that this and other anionic polymers bind to positively charged residues of proteins, such as lipoproteins (Nishida and Cogan, 1970; Krumbiegal et al., 1988).

Moreover, dextran sulfate has been shown to inhibit the attachment of poliovirus to monkey kidney cells (Bengtsson, 1965). It has been shown recently that this polymer is able to suppress the binding of human immunodeficiency virus (HIV) to CD4 proteins of their host cells (Ito et al., 1987; Ueno and Kuno, 1987). Since then, several studies on the inhibitory effects of dextran sulfate and related compounds on HIV have been reported (Bagasra and Lischner, 1988; Baba et al., 1988; Chang et al., 1988; Mitsuya et al., 1988; Ito et al., 1989). Some sulfated polysaccharides (heparin etc.) have also been shown to have a close relationship between anti-HIV activity and antithrombin activity (Baba et al., 1988, 1989). It has recently been demonstrated that dextran sulfate inhibits the fusion of Influenza A virus (Lüscher-Mattli and Glück 1990), and Sendai virus (Arnold et al., 1990) with liposomes. Also, a preliminary study on the inhibitory effect of dextran sulfate of MW 100 K on Sendai virus-erythrocyte ghost fusion has been reported (Ohki et al., 1991).

In this paper, we have extended and clarified the effect of sulfated polymers (dextran sulfate of different molecular weights, heparin and chondroitin sulfate) on the fusion of Sendai virus with erythrocyte ghosts. In addition, we

have studied (1) the effect of dextran sulfate on the binding of virions to erythrocyte ghosts using a fluorescence method, and (2) the adsorption of dextran sulfate on virion and erythrocyte ghost surfaces by use of the microelectrophoresis technique. These studies are not part of the previous paper (Ohki et al., 1991).

## Materials and Methods

*Sendai virus*

The Cantell strain Sendai virus was grown in fertilized chicken eggs, and purified from allantoic fluid according to the published method (Cantell and Hirvonen, 1981; Haywood, 1974) and suspended in 0.1 M phosphate buffer, pH 7.0. Purified virus was aliquoted and stored at −40°C.

The fusion of Sendai virus with erythrocyte ghosts was studied by use of the $R_{18}$ dequenching assay (Hoekstra et al., 1984). $R_{18}$ (Octadecylrhodamine) was obtained from Molecular Probes (Eugene, OR). Virus corresponding to 1 mg of virus protein suspended in 1 ml of 0.15 M NaCl/10 mm Tricine, pH 7.4 was labelled with $R_{18}$ by rapid injection of an ethanoic solution of $R_{18}$ (approximately 1% of virus lipid). After incubation at 24°C for 1 h, the unincorporated $R_{18}$ was removed from the virus suspension by filtration on a Sephadex G-75 column (Hoekstra et al., 1984). At this concentration of $R_{18}$ incorporated in the virus envelope, $R_{18}$, fluorescence was about 90 quenched.

*Human erythrocyte ghosts*

The fresh human blood (type A) obtained from a healthy individual was washed 3 times with 120 mM KCl/30 mM NaCl/10 mM potassium phosphate, pH 8.0. About 1 ml of packed erythrocytes was used to prepare ghost cells according to the method of Steck and Kant (1974) with some modifications (Leonards and Ohki 1983). The lysed cells were suspended in 10 vols. of 120 mM KCl/30 mM NaCl/10 mM phosphate, pH 8.0 and placed on ice for 15 min. In some cases, the buffer solution contained 2% (w/w) bovine serum albumin. The ghosts were resealed at 37°C for 45 min in the above KCl buffer containing 2 mM $MgCl_2$. The ghost cell suspension was washed with 0.15 M KCl/5 mM sodium-phosphate, pH 7.4 three times at 22,000 × *g* for 20 min at 4°C and the final ghost pellet was resuspended at a concentration of about 1.5 mg protein/ml, in 0.15 M NaCl/10 mM trycine, pH 7.4 (0.15 M NaCl buffer) at 0°C.

*Fusion assay*

The excitation and emission maxima of $R_{18}$ occur at the wave lengths 560 nm and 585 nm, respectively. When Sendai virus envelopes labelled with $R_{18}$ fuse with a unlabelled host cell and the fluorescent probe $R_{18}$ is diluted into the fused host cell membrane, there is an increase in fluorescence intensity as a result of dequenching of the probe. This change is related to the extent of fusion between the Sendai virions and the host cells (Hoekstra, 1984).

The fusion assay was done as follows: 20 $\mu$l of Sendai virus stock solutions ($\sim$20 $\mu$g protein) and 100 $\mu$l of erythrocyte ghosts stock solution ($\sim$150 $\mu$g proteins) were mixed with 100 $\mu$l of 0.15 M NaCl buffer solution of a given pH and incubated at 0°C for 10 min and then the virus–erythrocyte ghost mixture, 'the fusion mixture', was suspended in 2 ml warm NaCl buffer (37°C). The dequenching signal of the fusion mixture was monitored at 37°C with a fluorimeter (Perkin-Elmer, LS5) equipped with temperature-controlled cell holders.

Sendai virus labelled with 1% of $R_{18}$ to the lipids of virus preparation showed the quenching of 90% of the maximum fluorescence. The fluorescence signal of the quenched state is denoted by $I_o$. As the fusion of the virus with erythrocyte ghosts proceeded, the increase of fluorescence signal (I) was observed at 585 nm. When 0.2% of Triton X-100 was added to such a suspension, the fluorescence signal reached a maximum value ($I_t$) at 585 nm, probably a result of complete dispersion of $R_{18}$ into micellar forms. This state was considered as 100% fusion of the virus-erythrocyte ghost mixture. The extent of fusion (F) was defined as

$$F = (I - I_o)/(I_t - I_o) \times 100$$

The effect of various anionic polymers on the virus–erythrocyte fusion was examined in the following manner; first, the $R_{18}$-labelled Sendai virus (20 $\mu$g protein) and a certain amount of anion polymers in 100 $\mu$l of 0.15 M NaCl at a given pH were mixed, incubated for 5 min on ice (0°C) and then the erythrocyte ghosts (150 $\mu$g proteins) were added to the mixture and incubated for another 10 min on ice. The incubated mixtures were then suspended in 2 ml of the warm NaCl buffer solution (37°C) at a given pH. The time course of dequenching of $R_{18}$ at 37°C was followed with the fluorimeter. The effect of alternative incubations of virus and erythrocyte ghosts with dextran sulfate were examined. Erythrocyte ghosts and dextran sulfate were mixed first, incubated for 5 min on ice and then the virus was added, followed by incubation for 10 min on ice. Another preincubation was done by mixing the virus and erythrocyte ghosts for 10 min on ice and then dextran sulfate was added to the mixtures for 5 min on ice. These mixtures were then suspended in 2 ml of the warm NaCl buffer solution (37°C) and the dequenching signal of $R_{18}$ was monitored. Dextran sulfates of MW 8 K and 40 K were obtained from ICN Biochemical (Ohio) and that of MW 100 K was from Serva Biochemicals (Westbury, NY). The experiments using other sulfated polymers such as Heparin (MW 15,000) and chondroitin sulfate C (MW 60,000) both of which were obtained from Calbiochem Co., were done in a similar manner to those using dextran sulfate. Dextran 10K was obtained from Fluka Co.

*Binding of Sendai virus to erythrocyte ghosts* Binding or adhesion between Sendai virus and erythrocyte ghosts was examined by measuring the $R_{18}$ fluorescence-labelled Sendai viruses which were adsorbed onto the erythrocyte

ghosts. Virus was allowed to bind to erythrocyte ghosts at 0°C for 10 min in 0.15 M NaCl buffer at pH adjusted to various values. The suspension was centrifuged at 20,000 × *g* with a table-top Eppendorf centrifuge (Model 5414) for 2 min at 4°C. The supernatant and the erythrocyte ghost pellet were separated and the pellet was suspended in 0.15 M NaCl buffer solution. Both the supernatant and the pellet suspension were treated with 0.5% Triton (final concentration). The fluorescence (at 585 nm) of $R_{18}$ was measured to determine the distribution of virions in the two phases between the supernatant and the pellet. The effects of dextran sulfate and heparin on binding were studied by the incorporation of the polymers into the incubation buffer prior to and after addition of the erythrocyte ghosts as described above.

*Aggregation among Sendai virions and erythrocyte ghosts* Aggregation of virions or erythrocyte ghosts induced by the anionic polymers was examined by measuring the turbidity of respective suspensions. For virions, a small amount (50 μg protein) of the virus stock solution was suspended in 2 ml of the warm 0.15 M NaCl buffer solution (37°C) at various pH values (7.4, 6.5, 5.5, 4.5, 3.8), and, at each pH, various amounts of the sulfated polymers (1 μg/ml–0.25 mg/ml) were added to the virion suspension solution at 2-min intervals. Alternatively, the same amount of virus and various amounts of the sulfated polymers were suspended in 2 ml of the warm 0.15 M NaCl buffer (37°C) at pH 7.4 and then the pH of the suspension solution was changed with the citrate buffer (10 mM) or HCl to a proper value and the turbidity of the suspension was measured at various pHs. For erythrocyte ghosts, an amount equivalent to 150 μg protein of the stock erythrocyte ghosts was suspended in 2 ml of the warm NaCl buffer (37°C) having various pH values as above and the similar experiments as the virus with respect to anionic polymer concentration and pH of the solution were performed. The turbidities of the suspensions were measured at 450 nm with a dual beam spectrophotometer (Hitachi 100–60) equipped with a temperature-controlled cell arrangement.

*Cell electrophoresis*

Electrophoretic mobility measurements of virus and erythrocyte ghosts were performed using Melvern Zeta-sizer IIC instrument with He-Ne Laser light source (632.8 nm). Sendai virus (100 μg protein/ml) and erythrocyte ghosts (150 μg protein/ml) were suspended in 0.15 M NaCl buffer solution/pH 7.4 at 37°C.

All experiments were done at 37°C. The protein assay for Sendai virus and erythrocyte ghosts was done by the method of Bradford (1976) (BioRad Protein Assay) using bovine serum albumin (BSA) as standard.

## Experimental results

*Fusion experiments*

Fig. 1A shows a typical time course of $R_{18}$ fluorescence measured at 585 nm

for a suspension of the fusion mixture which consisted of 20 μg protein of the $R_{18}$-labelled Sendai virus and 150 μg protein of the erythrocyte ghost in 2 ml of buffer. In this experiment, Sendai virions and erythrocyte ghosts were mixed and incubated for 10 min and suspended; the mixture in the warm NaCl buffer was at pH 7.4 (detailed procedures are given in the Material and Methods section). Most fusion reactions seemed to saturate after 45 min to 1 h. At pH 7.4, the magnitude of the dequenched signal resulting from fusion was found to be about 30% of the maximum. The extent of virus–erythrocyte ghost fusion measured at 45 min after the mixture was suspended in the warm (37°C) NaCl buffer solution varied in the range of 28–32% fusion maximum for different batches of purified virus stocks. The value of 30% fusion was an average value over several experiments. For the erythrocyte ghosts with or without albumin in the ghost preparation process, there was no appreciable difference in fusion of virus with erythrocyte ghosts. The suspension of $R_{18}$-labelled virus alone did not show any increase in fluorescence at 585 nm for an extended period of time (2–3 h) under the same conditions employed for the above fusion experiment.

The effect of dextran sulfate on fusion of the virus with erythrocyte ghosts was examined under the same fusion conditions except for the addition of dextran sulfate. The order of mixing the samples was as follows: Sendai virions and a certain amount of dextran sulfate were mixed first and then erythrocyte ghosts were added (detailed procedures are given in the Materials and Method section). The addition of dextran sulfate suppressed fusion of Sendai virus with erythrocyte ghosts. A typical time course of such a suppressed fusion reaction at pH 7.4 is shown in Fig. 1A'. The suppression was dependent on the dextran sulfate concentration as shown in Table 1. The higher the concentration of dextran sulfate, the greater the suppression. However, at approximately 0.5 mg/ml of dextran sulfate, the effect of suppression reached saturation. The effect of the order of addition of dextran sulfate to the fusion mixture on fusion was

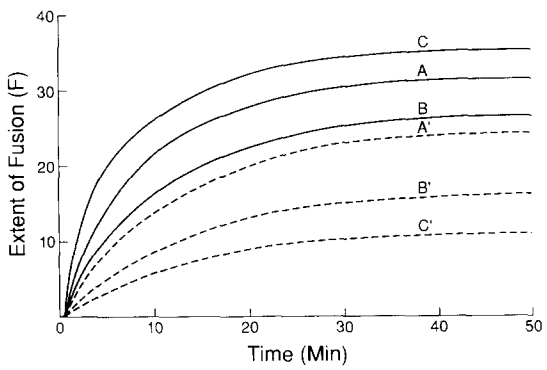


Fig. 1. Typical time course of $R_{18}$ fluorescence measured at 585 nm (at the maximum in emission spectrum) for the fusion mixtures suspended in warm (37°C) 0.15 M NaCl buffer solutions of various pHs ((A) pH 7.4, (B) pH 5.5, and (C) pH 3.8). The final concentration of cells: Sendai virus = 10 μg protein/ml, human erythrocyte ghost = 75 μg protein/ml, without dextran sulfate and with 0.5 mg dextran sulfate (MW 40 K)/ml. A, B, and C: no dextran sulfate, A', B', and C': in the presence of dextran sulfate.

TABLE 1

Extent of fusion of Sendai virus (~20 μg protein) and human erythrocyte ghosts (150 μg protein) suspended in 2 ml of 0.15 M NaCl/10 mM tricine pH 7.4 at 37°C, and the effect of dextran sulfate on fusion

| Dextran sulfate | % fusion maximum at 45 min | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 0.005 | 0.05 | 0.1 | 0.5 | Conc. 1.25 (mg/ml) |
| (MW) | | | | | | |
| $1 \times 10^5$ | 29.4 ± 0.5 | 26.1 ± 0.5 | 24.2 ± 0.4 | 23.5 ± 0.5 | 23.0 ± 0.5 | 22.5 ± 0.6 |
| $4 \times 10^4$ | 29.4 ± 0.5 | 26.4 ± 0.5 | 24.6 ± 0.5 | 23.5 ± 0.6 | 22.8 ± 0.6 | 23.0 ± 0.7 |
| $8 \times 10^3$ | 29.4 ± 0.5 | 27.0 ± 0.5 | 25.4 ± 0.5 | 24.6 ± 0.6 | 23.7 ± 0.5 | 24.0 ± 0.6 |

The values are reported as mean ± S.D.

examined. The following order, mixing virus and dextran sulfate first, and then the addition of erythrocyte ghosts, alternatively mixing erythrocyte ghosts and dextran sulfate first, and then the addition of the viruses, did not show any appreciable difference in the results. However, when erythrocyte ghosts and virus were mixed first and incubated for 10 min on ice (0°C), and then dextran sulfate was added to the mixture and incubated for 5 min on ice, the extent of fusion observed at 37°C. In this case, fusion was less suppressed than those for the other two cases. This experiment suggests that dextran sulfate appears to primarily inhibit the process of adhesion between virus and erythrocyte ghosts. However, a minor effect on the fusion process cannot be excluded from these data. The higher the molecular weight of dextran sulfate, the slightly more effective was the suppression of fusion. Another experiment, the effect of non-sulfated dextran on the same fusion system was examined. There was no effect on the fusion up to the concentration of dextran (10K) of 1 mg/ml. Heparin and chondroitin sulfate C also suppressed the fusion of the virions with the erythrocyte ghosts. The effectiveness of suppressing the viral fusion among the anionic polymers at pH 7.4 is in the order of dextran sulfate (40 K) > heparin (15 K) $\gtrsim$ chondroitin sulfate C (60 K) from the application of the same amount (weight/ml) of sulfated polymers.

Another series of fusion experiments was performed to examine the effect of pH on such fusion reactions. Various pH values (pH 7.4, 6.5, 5.5, 4.5, and 3.8) were used in the reaction mixtures. For some experiments, sodium citrate was used as buffer for the lower pH solutions of 5.5, 4.5 and 3.8. The experiments for lower pH were also done using the trycine-buffered solution by adjusting pH with HCl just before the experiments were performed. Results from each experiment were identical within the experimental errors. The intensity of the $R_{18}$ fluorescence emission for the $R_{18}$-labelled virions without fusion or the probe dilution was not affected by the change in pH (3.5–8.0) of the suspension solution. Fig. 2 shows the extent of fusion at 45 min in the presence and the absence of various sulfated polymers (0.5 mg/ml) at different pH for the case of the following order of mixing: virion and anionic polymers were mixed first and then erythrocyte ghosts were added to the mixture as detailed in the Materials

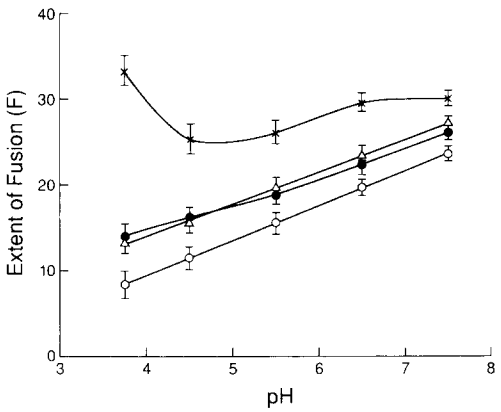


Fig. 2. The extent of fusion of Sendai virus with erythrocyte ghosts at 40 min after the fusion mixture was suspended in the warm (37°C) 0.15 M NaCl buffer solution at various pHs. ×, in the absence of anionic polymers; ○, in the presence of 0.5 mg dextran sulfate (MW 40 K)/ml; ●, in the presence of 0.5 mg heparin/ml; △, in the presence of 0.5 mg chondroitin sulfate/ml. The error bar for each data point refers to the standard deviation (S.D.).

and Methods section. In the absence of anionic polymers the extent of fusion was unchanged as the pH was lowered from pH 7.4 to 6.5. As pH was further lowered from 6.5 down to 4.5, the extent of fusion was slightly reduced. However, at pH lower than 4.5, the extent of fusion increased greatly. The effect of anionic polymers on suppression of fusion increased gradually as pH was lowered. At pH 3.8, the rate and extent of suppression of fusion by dextran sulfate increased greatly and the inhibition of fusion by dextran sulfate (MW 40 K, 0.5 mg/ml) reached approximately 80% (see Fig. 2). Over the pH range (7.4–3.8) examined, the order of effectiveness to suppress fusion was also dextran sulfate (MW = 40 K) > heparin (MW = 15 K) $\gtrsim$ chondroitin sulfate C (MW = 60 K) (see Fig. 2).

In order to examine the mode of interaction of the above sulfated polymers with cell surfaces, which may have an effect on fusion between virion and erythrocyte ghost cells, the effect of their polymers on binding or adhesion between virions and erythrocyte ghosts was examined by use of a fluorescence method. First, the erythrocyte ghosts (100 $\mu$l $\sim$ 150 $\mu$g protein) and $R_{18}$-labelled Sendai virus (20 $\mu$l $\sim$ 20 $\mu$g protein), which were the same amounts used in the fusion experiments were mixed with 100 $\mu$l of 0.15 M NaCl buffer solution with and without dextran sulfate (0.5 mg/ml) at a given pH (pH 7.4 or pH 5.0) at 4°C. At this stage the aggregation of virions to erythrocyte ghosts occurred. However, such aggregates or bound complexes did not show fusion at 4°C. Then the virions bound with erythrocyte ghosts and free virions were separated by centrifugation. Each portion was suspended in the NaCl buffer solution of a given pH (pH 7.4 or 5.0) and then the contents in each suspension were lysed by Triton X-100 to detect the amounts of $R_{18}$ in each cell suspension. The following were the results from such studies (Table 2): in the

TABLE 2

Effect of dextran sulfate (0.5 mg/ml) on binding of Sendai virus to erythrocyte ghosts and comparison between the extents of binding at 0°C and fusion at 37°C in 0.15 M NaCl buffer at the respective pHs

| | pH.4 | | | pH 5.0 | | |
|---|---|---|---|---|---|---|
| | Control (no DS) | DS (40 K) | DS (100 K) | Control (no DS) | DS (40 K) | DS (100 K) |
| $\frac{Virus^{pellet}}{Virus^{pellet} + Virus^{sup.}}$ | 0.83 ± 0.03 | 0.70 ± 0.02 | 0.65 ± 0.03 | 0.91 ± 0.03 | 0.42 ± 0.02 | 0.28 ± 0.03 |
| Reduction of binding | 0% | 16 ± 2% | 22 ± 2% | 0% | 55 ± 3% | 71 ± 3% |
| Reduction of fusion | 0% | 23 ± 3% | 32 ± 3% | 0% | 62 ± 3% (pH 4.5) | 82 ± 4% (pH 4.5) |

The results are given as mean ± S.D.

absence of dextran sulfate the ratio of the amount of virion bound to erythrocyte ghosts to that of the total virions was 0.83 at pH 7.4 and 0.91 at pH 5.0. Dextran sulfate suppressed such binding of virions with erythrocyte ghosts; at pH 7.4, there was a 16% decrease in binding in the presence of 0.5 mg/ml of dextran sulfate of molecular weight 40 K and a 22% decrease for the molecular weight 100 K. At pH 5.0, a 55% decrease in binding in the presence of 0.5 mg/ml of dextran sulfate of molecular weight 40 K and a 71% decrease for that of the molecular weight 100 K were observed, respectively. The results are given in Table 2. These results compared well with the reduction of fusion of Sendai virions to erythrocyte ghosts with dextran sulfate described earlier (see also Table 2).

In order to examine the interaction of the anionic polymers with either Sendai virus or erythrocyte ghosts, aggregation of virus and of cells in the presence of the polymers was also examined as a function of concentration of the polymers and pH of the solution. The anionic polymer was added

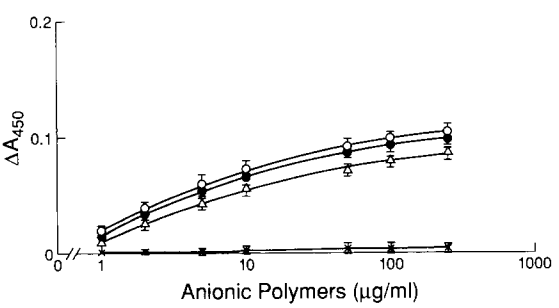


Fig. 3. Changes in turbidity ($A_{450}$) of Sendai virus (50 μg/ml) or erythrocyte ghosts (75 μg/ml) in 0.15 M NaCl buffer solution (37°C and) at pH 4.5 with respect to various concentrations of anionic polymers. For Sendai virus: ○, dextran sulfate (40 K); ●, heparin; △, chondroitin sulfate. For erythrocyte ghosts: ×, dextran sulfate (40 K). (The bar refers to S.D.).

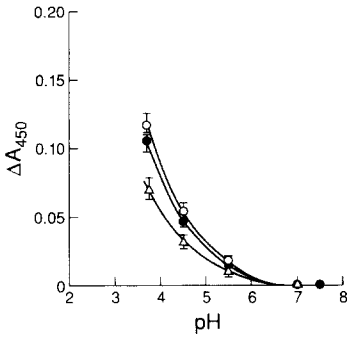


Fig. 4. Turbidity increases ($A_{450}$) of Sendai virus suspension (50 $\mu$g/ml of 0.15 M NaCl buffer) in the presence of 0.25 mg anionic polymers/ml at various pHs of the suspension solutions (37°C) with respect to those in the absence of the anionic polymers: ○, dextran sulfate (40 K); ●, heparin; △, chondroitin sulfate. (The bar refers to S.D.)

successively to the virus or erythrocyte ghost suspensions at a given pH in the amount of 1 $\mu$g/ml to 0.25 mg/ml. As the polymer concentration increased, the turbidity of the Sendai virus suspension increased but this was not observed for the erythrocyte ghost suspension. At neutral pH 7.4, there was a little increase in turbidity of virus suspension when the polymer concentration was increased. The change in turbidity with increase in dextran sulfate concentration was greater at lower pH. The results obtained at pH 4.5 are shown in Fig. 3. The turbidity change of the virus suspension due to the addition of the anionic polymers was pH-dependent. As the pH was lowered, the turbidity change increased. The changes of turbidity in the Sendai virus suspension of 0.15 M NaCl containing the anionic polymers of 0.25 mg/ml with the change in pH are shown in Fig. 4. In the absence of the anionic polymer there was no increase in

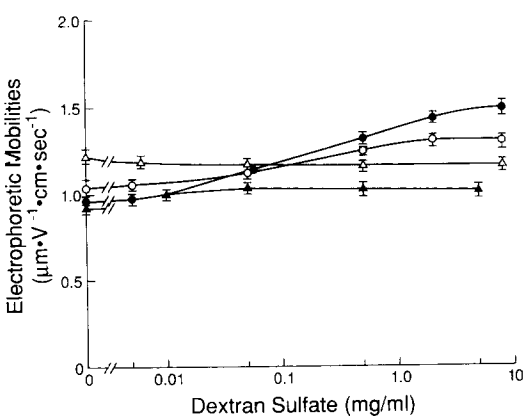


Fig. 5. Electrophoretic mobilities of Sendai virus (100 $\mu$g proteins/ml) and human erythrocyte ghosts (150 $\mu$g proteins/ml) suspended in 2 ml of 0.15 M NaCl/10 mM tricine (37°C) as a function of dextran sulfate (MW 40 K) concentration. ○, Sendai virus (pH 7.4); △, human erythrocyte ghosts (pH 7.4); ●, Sendai virus (pH 5.0); ▲, human erythrocyte ghosts (pH 5.0). (The bar refers to S.D.)

turbidity for Sendai virus suspension in the range of pH 3.8–7.4. The erythrocyte ghost suspension showed some increase in turbidity at pH 4.0. However, there was no increase in turbidity of the erythrocyte ghost suspension at various concentrations of dextran sulfate in 0.15 M NaCl at pH in the range of 4.5–7.4. Similar results were obtained for all anionic polymers tested (dextran sulfate (8 K, 40 K and 100 K) heparin (15 K) and chondroitin sulfate C (60 K)). However, the extent of aggregation of virions was of the same magnitude for dextran sulfate (40 K) and heparin (15 K) but was less for chondroitin sulfate (60 K).

The electrophoretic mobilities of Sendai virus and erythrocyte ghosts were both negative in sign in 0.15 M NaCl buffer solution at pH 7.4 and 5.0. Their values were $1.02 \pm 0.05$ and $1.22 \pm 0.05$ $\mu m\ s^{-1}\ cm\ V^{-1}$, respectively at pH 7.4 and $0.91 \pm 0.05$ and $0.95 \pm 0.5$, respectively at pH 5.0 (see Fig. 5). The electrophoretic mobility of Sendai virus increased with the concentration of dextran sulfate (40 K). At concentrations greater than 5 mg/ml, the electrophoretic mobility of virions saturated, which was about 1.30 $\mu m\ V^{-1}\ cm\ s^{-1}$ for pH 7.4 and 1.47 $\mu m\ V^{-1}\ cm\ s^{-1}$ for pH 5.0. The increase of 0.28 $\mu m\ V^{-1}\ cm\ s^{-1}$ in electrophoretic mobility from that in the absence of dextran sulfate at pH 7.4, corresponds to the increase in $\zeta$-potential of about 3.9 mV in magnitude. The increase in electrophoretic mobility at pH 5.0 was 0.56 which corresponds to the increase in $\zeta$-potential of about 7.8 mV in magnitude. On the other hand, the electrophoretic mobility of erythrocyte ghosts remained unchanged in the presence of dextran sulfate concentration up to 5 mg/ml in the suspension solution. These results indicate clearly that the dextran sulfate is adsorbed onto the virus membrane but not onto the erythrocyte ghost surface. Dextran (10 K) of 1 mg/ml did not affect both mobilities of the virions and the erythrocyte ghosts at the same condition as the above experiments.

## Discussion

The characteristics of fusion of Sendai virus with erythrocyte ghosts in the absence of dextran sulfate followed closely previously published reports by other workers (Hoekstra and Klappe, 1986).

All anionic polymers used in this experiment suppressed the fusion of Sendai virus with erythrocyte ghosts. These results are similar to the earlier work on the effect of dextran sulfate on the fusion of influenza virus with liposomes (Lüscher-Mattli and Glück, 1990) and that (MW 100 K) of Sendai virus with liposomes (Arnold et al., 1990) and with erythrocyte ghosts (Ohki et al., 1991). The lower the pH of the cell suspension solution, the larger was the suppressive effect. The effect of the suppression was dependent on the concentration of the polymers. The higher the concentration, the more effective was the suppression. However, the effect tended to saturate at a higher concentration (e.g. 0.5 mg/ml: dextran sulfate of 100 K) for each polymer.

The turbidity of the Sendai virus suspension increased in the presence of the

anionic polymers as the pH of the solution was lowered. Similar increases in turbidity were also observed for an erythrocyte ghost suspension but the effect was of much less magnitude compared to that of the Sendai virus suspension. The order of effectiveness among these polymers was dextran sulfate (40 K) > heparin (15 K) >, chondroitin sulfate (60 K). The negative charged group per unit monomer are 3 for dextran sulfate, 1.5–2 for heparin and 1 for chondroitin sulfate, respectively. The effect of these anionic polymers on fusion and aggregation of the cells may thus be related to the number of charged sulfate groups per monomer unit.

Furthermore, the binding studies of virions with erythrocyte ghosts (Fig. 3 and Table 2) and the electrophoretic mobility measurements of virions (Fig. 5) clearly indicate that dextran sulfate binds to virus surfaces but not to the erythrocyte ghosts and reduces the amount of bound virus to the erythrocyte ghosts at 4°C (Table 2). The suppression of binding is pH-dependent and somewhat molecular weight-dependent. These dependences parallel those of virus-erythrocyte ghost fusion. These results indicate that the negatively charged polymers may bind with positively charged groups on the virion membrane surfaces, possibly with the proteins of the Sendai virus envelope to cause aggregation of virus, even though the surface of Sendai virus membrane has an overall negative charge density according to our electrophoretic mobility measurements. The negative surface charge density of Sendai virus measured by use of a different experimental method was reported earlier (Haywood, 1974). When the pH of the solution is lowered from neutral pH, the negative charge residues of the protein will be neutralized according to their pK values, while the positive charges on the residues may remain unaffected. Consequently, the molecular surfaces of proteins as a whole become more positively charged. This tendency may increase the binding or the adsorption of the anionic polymers to the virion surface since the sulfate groups are negatively charged. This interpretation was supported by our binding studies of Sendai virions with erythrocyte ghosts and the electrophoretic mobility measurements of Sendai virions, the results of which have been discussed above. Table 2 shows that dextran sulfate suppressed the binding of Sendai virus to erythrocyte ghosts. The parallel results of suppression of dextran sulfate for both fusion and adhesion of Sendai virion–erythrocyte ghosts indicates that the principal effect of these anionic polymers is likely to be the suppression of the adhesion process between the virion and erythrocyte ghosts after these anionic polymers are adsorbed on the virion surfaces. Studies on binding between the positively charged amino groups of other proteins and dextran sulfate have been reported (Nishida and Cogan, 1970; Krumbiegel et al., 1988). The suppression effect of the sulfated polymers on viral fusion may be due not only for the negative charged groups of the polymers to bind to the positively charged sites of the proteins and to reduce the electrostatic binding between virions and erythrocyte ghosts, but also may be due to the steric hindrance of a massive hydrophilic molecule adhered to the virion surface. The observation that the anionic polymers of higher molecular weight resulted in slightly

stronger suppression of the fusion of Sendai virus–erythrocyte ghosts than that of lower molecular weight, also support this possibility. This adherence of large hydrophilic polymers may serve not only sterically to interfere with the close approach of two interacting membranes (Maroudas, 1975) but may also interfere with the close approach of two membranes by exerting the repulsive forces due to surface hydration (LeNeveu et al., 1976; Marcelja and Radic, 1976). These effects can also occur with the surface of the erythrocyte ghosts. However, our observations show that the major effects of these anionic polymers was with Sendai virus surfaces. This conclusion is also supported by the finding that the fusion of Sendai virus with phosphatidylserine vesicles is strongly inhibited by dextran sulfate (Arnold et al., 1990). In this case, the only site of binding of dextran sulfate is the virus surface since dextran sulfate does not show any interaction with phosphatidylserine membranes. Another possibility for the suppression of both binding and fusion of virions to erythrocyte ghosts could be due partly to the aggregation among virions induced by the anionic polymers; such aggregation among virions would simply reduce the amount of binding of the virions to erythrocyte ghosts and therefore reduce the extent of their fusion.

Further studies are necessary to determine which of the proteins (HN or F) of the viral envelope surface interacts more preferentially with these anionic polymers. The present experiments have demonstrated that the anionic polymers clearly suppress the fusion of Sendai viruses with erythrocyte ghosts and the interaction mode seems to be electrostatic between sulfate groups of the polymers and the positively charged sites of the protein surfaces on the viral envelope membrane. Other physicochemical factors (steric as well as hydrophilic) of the anionic polymer and the aggregation among virions due to the anionic polymers may also contribute to decrease in fusion between Sendai virus and erythrocyte ghost membranes.

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