Binding of herpes simplex virus to cellular heparan sulphate, an initial step in the adsorption process

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It has been suggested that heparan sulphate has a receptor function in the initial phase of the attachment of herpes simplex virus (HSV) to cells. We have studied the influence of glycosaminoglycans on cell adsorption of, and plaque formation by, HSV-1 and HSV-2, with regard to the role of saccharide structure, chain length and charge density. Heparin and highly-sulphated heparan sulphate (1-5 sulphate groups/disaccharide unit), but neither chondroitin sulphate nor dermatan sulphate, were found to compete with the cellular receptor for attachment of HSV. Heparan sulphate preparations of low sulphate content (0.5 and 0.7 sulphate groups/disaccharide unit) failed to show any significant interaction with HSV. Oligosaccharides generated by partial deaminative cleavage of heparin were used to determine the minimum molecular size required for the binding of virus; the smallest oligosaccharide which reacted with HSV was composed of 10 monosaccharide units. The importance of charge density was demonstrated more directly by subfractionation of the heparin dodecasaccharide fraction by anion-exchange HPLC. The virus-binding capacities of the four resulting dodecasaccharide subfractions increased from the least sulphated to the most heavily sulphated fraction. The results reported are discussed in relation to virus–receptor interactions involved in the attachment of HSV, including the reported binding of HSV to the fibroblast growth factor receptor.

Introduction

Herpes simplex virus (HSV) binds to heparin and the related glycosaminoglycan, heparan sulphate (Nahmias & Kibrick, 1964; Takemoto & Fabish, 1964; Vaheri, 1964). Most mammalian cells carry heparan sulphate as a plasma membrane constituent (Kjellén et al., 1981; Höök et al., 1984; Lane et al., 1984) and it has been claimed that the anionic heparan sulphate can function as a cellular receptor in the initial surface interactions of HSV and the cell (WuDunn & Spear, 1989). This statement is supported by observations that enzymic digestion of the cell surface heparan sulphate reduces the binding of HSV to cells (WuDunn & Spear, 1989) and that the cationic aminoglycosides neomycin and polylsine block the binding of HSV to its cell receptor (Langeland et al., 1987, 1988). The blocking probably results from competition between the virus and the cationic compound. The inhibitory effect of different aminoglycosides on HSV infection parallels their cationic charge (Langeland et al., 1987).

Langeland et al. (1990) extended their studies by testing intertypic recombinants for sensitivity to neomycin and polylsine and located a region on the HSV-1 genome which includes the glycoprotein C (gC) gene and supposedly encodes viral proteins involved in the process of virus adsorption to the cell. However, recent reports by Muggeridge et al. (1990) and Kühn et al. (1990) suggest a role for gB and gC, as well as gD, in the binding and internalization of HSV-1. Kühn et al. (1990) suggest that the binding of gC, gB and gD occurs in a non-cooperative way.

Whether HSV-1 and HSV-2 interfere with each other at the cell surface level has been a matter of debate (Addison et al., 1984; Johnson & Ligas, 1988; Langeland et al., 1988; Vahlne et al., 1979; WuDunn & Spear, 1989) and it has not been determined whether the two types utilize the same or different receptor structures. Both HSV-1 and HSV-2 bind to heparan sulphate (WuDunn & Spear, 1989), but whereas neomycin reversibly blocks the binding of HSV-1 to cells, the attachment of HSV-2 is unaffected (Langeland et al., 1988), suggesting typespecific differences in the interactions of the virus and its cellular receptors.

It is assumed that the binding of HSV to heparan sulphate represents only a first step in a series of events ending with the delivery of the viral nucleocapsid to the cytosol (WuDunn & Spear, 1989). A recent report by
Kaner et al. (1990) demonstrates that HSV also binds to the high-affinity receptor of fibroblast growth factor (FGF). The binding of the virion to the FGF receptor seems essential for virus penetration of the plasma membrane. However, the mode of interaction of HSV and the FGF receptor is, as yet, unknown. Kaner et al. (1990) suggested that the virion reacts with extracellularly released FGF and that virion-bound FGF mediates the adsorption of virus to the receptor. Such a mechanism, they assume, would also account for the efficiency at which HSV can attach to heparan sulphate because the low-affinity FGF receptor is a heparan sulphate and FGF has a strong affinity for heparin.

In the present report we have characterized heparan sulphate involved in the initial interactions of HSV and cellular glycosaminoglycans. The effects of carbohydrate backbone structure, degree of sulphation and saccharide chain length are investigated.

**Methods**

**Viruses and cells.** The McIntyre and B4327UR strains of HSV-1 and -2, respectively, were used. Virus suspensions were freshly produced and cleared by centrifugation, first at 2000 g for 10 min and then at 15000 g for 5 min. The concentrations of infectious HSV-1 and HSV-2 were 5·1 × 10^6 and 2·4 × 10^6 p.f.u./ml, respectively; the particle p.f.u. ratio of the McIntyre strain was 3·5. The suspensions were diluted in MEM with Hanks' salts supplemented with 2% bovine serum albumin and used in the adsorption assays at a multiplicity of 50 p.f.u./cell.

In all experiments, green monkey kidney (GMK AH-1) cells were cultured in Eagle's MEM supplemented with 10% newborn calf serum. Cultures in conventional 5 cm plastic dishes were inoculated, overlaid with Hanks' salts supplemented with 2% bovine serum albumin and incubated for 1 h at 37 °C. The mixtures were then added to four wells and adsorption assays were performed. Virus mixed with the diluent of the glycosaminoglycan preparation and virus-free blanks, treated in parallel with the mixtures of virus and saccharide, served as controls.

To exclude the possibility that the presence of heparin in the virus inoculum interferes with the binding of MAbs B1C1 or O1C6 to cell-associated virus, heparin (200, 20 and 2 μg/ml) was added to cell culture plates pre-coated with HSV. Heparin did not influence the binding of the MAbs.

**Glycosaminoglycans.** Heparin (stage 14) from pig intestinal mucosa was obtained from Inoxel Pharmaceutical Division and purified by repeated precipitation with cetlypyridinium chloride from 1·2 M-NaCl, essentially as described (Lindahl et al., 1965). The product was fractionated by affinity chromatography on antithrombin-Sepharose, according to the method of Laurent et al. (1978). Polysaccharides in the effluent which either had not adsorbed to the affinity matrix or which emerged at a low salt concentration (< 0·7 M-NaCl) distinctly separated from the high-affinity material, were combined into one low-affinity pool. N-/O-desulphated and N-/O-desulphated, N-acetylated heparin were prepared as described (Jacobsen et al., 1979).

A preparation (A) of heparan sulphate (approx. 0·5 sulphate groups/disaccharide unit) isolated from human aorta was provided by W. Murphy, Melbourne, Australia. Two other preparations of heparan sulphate, derived from pig intestinal mucosa, were obtained from products of commercial heparin manufacture by fractional precipitation with cetylpyridinium chloride. Preparation B (0·7 sulphate groups/disaccharide unit) was obtained by precipitation from 0·4 M-NaCl, following the removal of material precipitable from 0·8 M-NaCl, and was purified further by digestion with bacterial chondroitin ABC lyase. Preparation C (1·5 sulphate groups/disaccharide unit) contained polysaccharide precipitable with cetylpyridinium chloride from 0·4 M-NaCl but not from 1·2 M-NaCl. Chondroitin sulphate (1·0 sulphate group/disaccharide unit), isolated from bovine nasal septa, was given by Ä. Wasteson, Linköping, Sweden. The dermatan sulphate preparation (1·3 sulphate groups/disaccharide unit) was donated by L. Rodén, Birmingham, Ala., U.S.A. The purification of this material involved elimination of heparin-related material by treatment with nitrous acid.

Heparin oligosaccharides were isolated after partial deaminative cleavage of the polysaccharide with nitrous acid and fractionated by gel chromatography. The procedure employed was essentially as described by Lane et al. (1984) except that affinity chromatography on immobilized antithrombin was omitted. All even-numbered components containing between four and 20 monosaccharide units were obtained in apparently homogeneous form.

A sample of the dodecasaccharide fraction was separated further on the basis of charge density (essentially reflecting sulphate content) by anion-exchange HPLC on a column (approx. 0·5 x 6 cm) of Mono Q HR 5/5 (Pharmacia), which was eluted with a linear salt gradient of...
Heparan sulphate as an HSV receptor

Fig. 1. Effects of heparin, chondroitin sulphate and dermatan sulphate on adsorption of HSV-1. Heparin concentrations of 2 (●), 20 (▲) and 200 (▲) µg/ml, 200 µg/ml chondroitin sulphate (□) and 200 µg/ml dermatan sulphate (■) were added to HSV-1, and the mixtures were incubated at 37 °C for 10 min; (□) control of virus only. Subsequently, GMK AH-1 cells were inoculated and incubated at room temperature for between 15 and 120 min. The amount of virus attached to cells was determined by ELISA. Each point shows the mean ± S.E.M. of four assays.

Fig. 2. Effect of the heparin icosasaccharide on the adsorption of HSV-2. The heparin icosasaccharide was produced by partial deaminative cleavage with nitrous acid followed by gel filtration. The icosasaccharide, at concentrations of 2 (●), 20 (▲) and 200 (▲) µg/ml, was mixed with HSV-2 and incubated at 37 °C for 10 min; (○) control of HSV-2 only. Subsequently, the mixtures were added to GMK AH-1 cells and incubated at room temperature for 15 to 120 min. The amount of virus attached to cells was determined by ELISA. Each point shows the mean ± S.E.M. of four assays.

0.3 M- to 2 M-NaCl in 0.05 M-sodium acetate, pH 4.0. The resulting distribution of poorly separated components was used to provide four arbitrarily pooled subfractions of variously sulphated dodecasaccharides (data not shown). Prior to use the saccharide fractions were desalted by passage through a column of Sephadex G-15, equilibrated with 10% aqueous ethanol. The saccharides were quantified by the carbazole reaction (Bitter & Muir, 1962) with glucuronolactone as a standard, assuming an average hexuronic acid content of 33% for the various preparations.

Results

Heparin but not chondroitin sulphate or dermatan sulphate competes with the cell receptor for HSV

Heparin was able to compete with cell receptors for HSV-1 in a dose-dependent fashion. Fig. 1 demonstrates that even at a concentration as low as 2 µg/ml, the addition of heparin resulted in an obvious reduction in the amount of cell-bound virus. Heparin with high or low affinity for antithrombin interacted equally well with both types of HSV (data not shown). Chondroitin sulphate and dermatan sulphate, on the other hand, showed no virus-binding capacity at concentrations of 200 µg/ml.

Analogous results were obtained in plaque reduction experiments (data not shown). Heparin significantly blocked infection at a concentration of 6-25 µg/ml, the lowest concentration tested, whereas neither chondroitin sulphate nor dermatan sulphate inhibited infection or plaque formation at a concentration of 50 µg/ml. These findings are consistent with those of WuDunn & Spear (1989), which were based on enzymic digestion of cell-surface glycosaminoglycans.

Importance of the molecular size of the competing saccharide

In a series of experiments we determined whether the capacity of heparin to inhibit the binding of HSV to cells, assayed by monitoring the competition between the glycosaminoglycan and the cell receptors for the virus, was proportional to the molecular size of the saccharide. By deaminative cleavage of heparin and subsequent fractionation of the products, preparations of even-numbered oligosaccharides, ranging from four to 20 monosaccharide units, were produced. We then assayed the virus-binding capacity of the individual preparations by cell receptor competition experiments; some preparations were assayed also for their ability to inhibit plaque formation. Experiments were performed with both HSV-1 and HSV-2.

Fig. 2 demonstrates the results obtained using the icosasaccharide and HSV-2. The virus-to-cell adsorption profiles of HSV-2 exposed to different concentrations of the saccharide suggested a dose-dependent inhibition pattern, similar to the findings reported with heparin and HSV-1 (Fig. 1). The smaller the saccharide chain length, the less efficiently the oligosaccharide preparation interacted with virus and competed with the cell receptor. As illustrated in Fig. 3(a), semilogarithmic plots fitted reasonably well to linear regressions. Assuming that binding of virus and competition with the
Fig. 3. (a) Semilogarithmic plots of HSV-2 adsorption in the presence of the heparin octa-, deca and dodecasaccharides. Heparin oligosaccharide preparations produced by deaminative cleavage followed by gel filtration were mixed in concentrations ranging from 3 to 200 μg/ml with HSV-2 (○, octasaccharide; ●, decasaccharide; △, dodecasaccharide; ▲, PBS control). The mixtures were incubated at 37 °C for 10 min and then added to GMK AH-1 cells which were further incubated at room temperature for 120 min. The amount of virus attached to cells was determined by ELISA. Each point shows the mean ± S.E.M. of four assays. (b) The affinity of HSV-1 and HSV-2 for heparin oligosaccharides of different saccharide chain length. Semilogarithmic plots such as those presented in (a) were constructed for the interaction of HSV-1 (○) and HSV-2 (●) with preparations of heparin oligosaccharides composed of eight to 20 monosaccharide units.

Plaque formation by HSV-1 after treatment of the virus with oligosaccharides containing six, 12, 16 and 20 saccharide units, respectively, demonstrated that the dodeca and larger saccharides inhibited infection and that this inhibition became more pronounced the larger the size of the oligosaccharide (data not shown).

Importance of charge density of the competing saccharide

Since a marked difference in virus-binding capacity between heparin and chondroitin or dermatan sulphate could conceivably be ascribed to differences in charge density, further experiments were performed using chemically modified heparin and various heparan sulphates as competing agents.

Desulphated heparin had virtually no virus-binding capacity. Essentially similar results were obtained with two preparations (A and B) of low-sulphated heparan sulphate (Fig. 4), containing 0-5 and 0-7 sulphate groups/disaccharide unit, respectively (see Methods). In contrast, preparation C, with 1-5 sulphate groups/disaccharide unit, had similar virus-binding capacity to that of intact heparin (Fig. 1 and 4), in accord with the notion that virus binding is promoted by an increasing charge density of the saccharide.

Further experiments were performed to define the importance of sulphate density in comparison to molecular size. The dodecasaccharide fraction recovered after
Density, preparation I having the lowest and preparation IV the highest sulphate content. Samples of 200 µg/ml of each preparation (O, preparation I; ●, preparation II; △, preparation III; ▲, preparation IV; □, control of virus only) were mixed with HSV-1 and the mixtures incubated at 37 °C for 10 min. Subsequently, the mixtures were added to GMK AH-1 cells and incubated at room temperature for 15 to 120 min. The amount of virus attached to the cells was assayed by ELISA. Each point shows the means ± S.E.M. of four assays.

Partial deaminative cleavage of heparin was separated into fractions of different charge densities by ion-exchange HPLC. Thus, four different fractions of the same dodecasaccharide chain but of different sulphate content were available. The results shown in Fig. 5 clearly demonstrate that the cell receptor-competitive capacity of the dodecasaccharide increased from fraction I, the least sulphated fraction, to fraction IV, the most heavily sulphated fraction.

Discussion

Cell attachment and penetration of HSV seem to be complex processes involving several viral envelope proteins. Binding to the cell, and fusion of the viral envelope and the plasma membrane of the cell by which penetration is accomplished (Fuller & Spear, 1987; Lycke et al., 1988; Morgan et al., 1968), includes activities of glycoproteins B, C, D and H (Cai et al., 1988; Campadelli-Fiume et al., 1990; Desai et al., 1988; Kühn et al., 1990; Ligas & Johnsson, 1988; Muggeridge et al., 1990), but it has not been established whether and how the glycoproteins interact and whether non-glycosylated envelope proteins are involved also in the attachment of the virion to the plasma membrane (Langeland et al., 1990; Spear, 1985).

HSV can infect a variety of mammalian cells of different species and the structures functioning as receptors are therefore probably common membrane constituents. In view of the finding that heparin can efficiently interfere with cell infection, heparin-related cell surface carbohydrates, such as heparan sulphate, have been proposed as possible HSV-binding receptors (WuDunn & Spear, 1989). However, several reported observations indicate that the binding of the virion to heparan sulphate probably represents only a step in the adsorption process (Kaner et al., 1990; Langeland et al., 1990; WuDunn & Spear, 1989). The most recent of these reports (Kaner et al., 1990) presents findings supporting the hypothesis that in addition to attachment to heparan sulphate, HSV-1 is bound to the high-affinity receptor of FGF and that this receptor is essential for the internalization of the nucleocapsid. As a plausible mechanism for the interactions between HSV-1 and the FGF receptor, Kaner et al. (1990) suggested that it is extracellularly released FGF bound to virions that mediates the adsorption of the virus to the FGF receptor; the presence of FGF on the viral envelope might also contribute to the efficiency with which HSV is bound to plasma membrane heparan sulphate (Kaner et al., 1990). The low-affinity FGF receptor is a transmembrane protein with an extracellular heparan sulphate moiety whereas the high-affinity FGF receptor belongs to the tyrosine kinase family (Blanquet et al., 1989).

Apparently, there is more than one pathway for HSV attachment and penetration, as has been suggested by Campadelli-Fiume et al. (1990).

WuDunn & Spear (1989) have reported that heparinase- and heparitinase-treated cells can be infected only at the highest concentrations of virus used, indicating a role for heparan sulphate in the adsorption of HSV to the cell surface. In agreement, Mettenleiter et al. (1990) have reported that the primary pathway of pseudorabies virus adsorption to cells also is dependent upon interaction between viral glycoprotein III (the HSV gC homologue) and a heparin-like cellular receptor, although the attachment of pseudorabies virus seems less sensitive to heparin than that of HSV-1 (Sawitzky et al., 1990).
length corresponding to at least 16 to 18 monosaccharide units. However, the structure of the carbohydrate backbone appears to be of some importance because virus binding was noted with a heparan sulphate preparation which contained 1-5 sulphate groups/disaccharide unit but not with a dermatan sulphate preparation which contained 1-3 sulphate groups/disaccharide unit. Our results suggest, moreover, that HSV-1 and HSV-2 both react with heparan sulphate with relatively marginal differences in oligosaccharide-bind- ing characteristics, as displayed in Fig. 3(b). A somewhat stronger affinity was noted for HSV-2 with more efficient binding, but it seems doubtful that this discrepancy would be sufficiently large to explain the observed differences in receptor affinity (Addison et al., 1984; Vahine et al., 1979) and sensitivity to competition by polycationic compounds (Langeland et al., 1988). However, as pointed out first by WuDunn & Spear (1989), attachment to heparan sulphate probably represents only the initial step in the anchoring of the virion to the plasma membrane. For the capture of the virion both the size of the saccharide chain and the charge density would be of importance. Binding presumably improves the possibility of the virion attaining contact with more selectively and specifically functioning receptor entities. Conceivably, binding to the high-affinity FGF receptor and/or the fusion reactions between the viral envelope and the plasma membrane could represent a second series of affinity-discriminating reactions and be responsible, for example, for the observed differences in the receptor requirements of HSV-1 and HSV-2.

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References


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