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Specificity and Affinity of Binding of Herpes Simplex Virus Type 2 Glycoprotein B to Glycosaminoglycans

RICHARD K. WILLIAMS* AND STEPHEN E. STRAUS

Medical Virology Section, Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20892

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Herpes simplex virus type 2 (HSV-2) interacts with cell surface glycosaminoglycans during virus attachment. Glycoprotein B of HSV-2 can potentially mediate the interaction between the virion and cell surface glycosaminoglycans. To determine the specificity, kinetics, and affinity of these interactions, we used plasmon resonance-based biosensor technology to measure HSV-2 glycoprotein binding to glycosaminoglycans in real time. The recombinant soluble ectodomain of HSV-2 gB (gB2) but not the soluble ectodomain of HSV-2 gD bound readily to biosensor surfaces coated with heparin. The affinity constants (K_ds) were determined for gB2 ($K_d = 7.7 \times 10^{-7}$ M) and for gB2ΔTM ($K_d = 9.9 \times 10^{-7}$ M), a recombinant soluble form of HSV-2 gB in which only its transmembrane domain has been deleted. gB2 binding to the heparin surface was competitively inhibited by low concentrations of heparin (50% effective dose [ED_{50}] = 0.08 μg/ml). Heparan sulfate and dermatan sulfate glycosaminoglycans have each been suggested as cell surface receptors for HSV. Our biosensor analyses showed that both heparan sulfate and dermatan sulfate inhibited gB2 binding (ED_{50} = 1 to 5 μg/ml), indicating that gB2 interacts with both heparin-like and dermatan sulfate glycosaminoglycans. Chondroitin sulfate A, in contrast, inhibited gB2 binding to heparin only at high levels (ED_{50} = 65 μg/ml). The affinity and specificity of gB2 binding to glycosaminoglycans demonstrated in these studies support its role in the initial binding of HSV-2 to cells bearing heparan sulfate or dermatan sulfate glycosaminoglycans.

The binding of herpes simplex virus (HSV-1 and HSV-2) envelope glycoproteins to heparin-like glycosaminoglycan side chains of cell surface proteoglycans occurs as a prelude to the entry of the virus into cells (25). Several types of experiments have been performed to investigate which glycoproteins of HSV-1 and HSV-2 are involved in binding to glycosaminoglycans. Two HSV-1 and HSV-2 envelope glycoproteins, gB (7, 12) and gC (7, 12, 30) bind heparin-agarose columns. Attachment of purified HSV-1 gC to cells is inhibited by exogenous heparin (28) and by pretreatment of cells with heparitinase (30). A role for HSV-1 gC in mediating virus attachment was shown in two types of experiments, one in which antibodies to HSV-1 gC inhibited HSV-1 adsorption (6, 28) and another in which mutant HSV-1 virions lacking gC were impaired in adsorption to cells (12, 19, 28). However, HSV-1 virions lacking both gC and gB are far more impaired in binding to cells than are virions that lack only gC, indicating that in HSV-1 gB also serves in virus attachment (11).

The recent discovery that an HSV-2 gC-negative mutant possesses normal cellular adsorption, entry, and plaque-forming efficiency indicates that although the HSV-2 gC protein binds heparin, it may not play the same virus attachment role as its HSV-1 counterpart (7). The receptor interactions of the HSV-2 gB protein are therefore especially important to the initial attachment of HSV-2 to susceptible cells. The B glycoproteins are highly conserved throughout the herpesvirus family, and it would appear that heparin binding by gB is also conserved among the herpesviruses, having been demonstrated, thus far, in a number of alphaherpesviruses and betaherpesviruses including pseudorabies (24), bovine herpesvirus 1 (2), and human cytomegalovirus (5), in addition to HSV-1 and HSV-2.

The structure of cell surface glycosaminoglycans is relevant to their recognition by HSV glycoproteins, since the length of the carbohydrate chains (21) and their degree of sulfation (9, 10) determine how effectively heparin inhibits HSV replication. That HSV-1 binding to membranes of several types of cells involves recognition of heparan sulfate, a heparin-like glycosaminoglycan, was shown in several experiments. Chondroitin sulfate A (chondroitin-4-sulfate) and dermatan sulfate did not inhibit (21), or marginally inhibited (1), the plaque-forming efficiency of HSV-1 in cells that express heparan sulfate on their membranes. HSV-1 adsorption was reduced by treatment of cells with enzymes which degrade heparan sulfate but not by enzymes which degrade chondroitin sulfates (31). Also, HSV-1 virions attach less well to cells which are defective in the biosynthesis of heparan sulfate (8). However, in heparan sulfate-deficient cells, HSV-1 plaque-forming efficiency is inhibited strongly by dermatan sulfate and somewhat by chondroitin sulfates A and C, leading to the hypothesis that one or more HSV-1 glycoproteins recognize dermatan sulfate and chondroitin sulfate A and C glycosaminoglycans on certain cells (1). These findings suggest that the relative affinities of HSV glycoproteins for different cognate glycosaminoglycan ligands may play an important role in HSV infections, tropism, and pathogenesis.

Developments in biosensor technology, in which surface plasmon resonance is quantitated as a measure of intermolecular binding (15, 16), have permitted the measurement of the real-time kinetics of binding of proteins to their ligands. Biosensor analyses of influenza virus hemagglutinin binding to sialic acid receptors (29) and of soluble intracellular cell adhesion molecule (ICAM) binding to human rhinovirus (4) have recently contributed to the understanding of the nature of these virus-receptor interactions. We investigated the interaction of recombinant HSV-2 gB (gB2) with heparin-like cell

* Corresponding author. Mailing address: Bldg. 10, Rm. 11N228, MSC 1888, National Institutes of Health, 9000 Rockville Pike, Bethesda, MD 20892-1888. Phone: (301) 496-5222. Fax: (301) 496-7383. E-mail: rwilliams@nih.gov.

surface receptors of MRC-5 fibroblasts. Then, using biosensor technology, we determined the binding kinetics and the specificity of the gB2 interaction with isolated glycosaminoglycans. These cell-free assays of the interaction of the gB2 with isolated glycosaminoglycans confirmed our findings regarding the binding of gB2 to live human fibroblasts.

MATERIALS AND METHODS

Effect of glycosaminoglycans on HSV-2 replication. Heparin and chondroitin sulfate A were compared for their relative abilities to inhibit HSV-2 growth. Confluent monolayers of Vero cells on six-well plates (Costar) were infected with HSV-2 strain 333 that had been preincubated for 15 min with heparin or chondroitin sulfate A at a range of concentrations in 0.5 ml of Eagle's minimal essential medium (EMEM) with 10% fetal calf serum, 25 μg of chlortetracycline per ml, 250 U of penicillin per ml, and 250 μg of streptomycin per ml. After a 60-min incubation at 37°C to allow virus adsorption, the cells were washed with maintenance medium (EMEM with 2% fetal calf serum, 25 μg of chlortetracycline per ml, 250 U of penicillin per ml, and 250 μg of streptomycin per ml) and then held at 37°C in maintenance medium with 0.05% human immunoglobulin G (IgG; Gammagard) for 2 days while plaques developed. At that time, the cells were stained with 1% crystal violet in 60% methanol–5% acetic acid–3.7% formaldehyde and the number of plaques in each well was counted.

Recombinant HSV-2 glycoproteins. Purified recombinant HSV-2 glycoproteins expressed in CHO cells were a gift of Rae Lyn Burke, Chiron Corp. These included soluble recombinant HSV-2 glycoprotein D (gD2) truncated before its transmembrane domain, the soluble recombinant ectodomain of HSV-2 glycoprotein B (gB2) (23, 27) truncated before its transmembrane domain, and another soluble recombinant HSV-2 gB form (gB2ΔTM) in which only the transmembrane domain was deleted, so that the gB ectodomain was fused directly to its cytoplasmic domain.

Localization of gB binding on the MRC-5 cell surface and its inhibition by glycosaminoglycans. HSV-2 gB2-binding sites were localized on the surface of MRC-5 cells by incubation of live cells with recombinant gB2 followed by immunofluorescent labeling. MRC-5 cells cultured for 2 days on glass coverslips were overlaid for 60 min at 4°C with complete media containing 0.3 μg of gB2 per ml and 0.02% sodium azide. The cells were washed three times with cold phosphate-buffered saline (PBS) and fixed for 5 min in 100% methanol for 10 min at −20°C. The cells were then incubated for 15 min in PBS with 10% fetal calf serum and stained with 1:100-diluted rabbit anti-HSV-2 (Dako) followed by fluorescein-conjugated goat anti-rabbit IgG (Cappel) in PBS with 1% normal goat serum. For inhibition studies, gB2 was preincubated in heparin or chondroitin sulfate A prior to binding of cells. Immunofluorescence was photographed with a 63× objective (Photomicroscope III; Zeiss), using color ASA 1600 film (Kodak) at equivalently timed exposures.

Effect on HSV-2 plaques by preincubation of cells with soluble HSV-2 glycoproteins. gB2, gB2ΔTM, and gD2 were tested for blocking of HSV-2 plaque formation. The soluble glycoproteins in serum-free EMEM were preincubated for 1 h at 4°C on Vero monolayers. HSV-2 (100 PFU per well) was allowed to adsorb for an additional 1 h in EMEM containing 1% fetal calf serum in the presence of the glycoproteins at 4°C. The Vero monolayers were then shifted to 37°C for 2 h in the continued presence of the glycoproteins. After this, the cells were washed in maintenance medium and overlaid for plaque formation as above.

Surface plasmon resonance measurement of HSV-2 glycoprotein binding. Measurements of glycoprotein binding were performed on a BIAcore biosensor system with CM5 biosensor chips (Pharmacia Biosensor) as previously described (15, 16). This system assesses the real-time binding of a protein to an immobilized ligand on a flow cell surface by measuring refractive index changes near a gold film surface to which the ligand is bound (15, 16). Glycosaminoglycans were immobilized on the biosensor surface as described by Mach et al. (22). The dextran-coated biosensor chips were equilibrated with HBS (10 mM HEPES [pH 7.5], 0.15 M NaCl, 3.4 mM EDTA, 0.05% Tween 20) and then activated with 0.05 M EDC [1-ethyl-3-(3-dimethylaminopropyl)carbodimide, 0.05 M *N*-hydroxysuccinimide). Streptavidin (Pierce) in 40 μl of 20 mM sodium acetate (pH 5.5) was pumped over the activated flow cell surface at 5 μl per min to allow coupling. Residual activated ester was blocked with 3 M Tris (pH 8.0). Glycosaminoglycans dissolved in PBS were biotinylated at amino groups by incubation for 1 h at room temperature with a molar excess of sulfo-*N*-hydroxysuccinimide-biotin (Pierce) (22). Excess biotin was removed on a Swift desalting column (Pierce). Biotinylated glycosaminoglycans (1 μg in 40 μl of PBS) were then immobilized on the streptavidin by injecting at 5 μl/min.

gB2, gB2ΔTM, and gD2 in HBS were applied to the biosensor to assess their interaction with heparin. Multiple binding experiments, with a single flow cell for each set of experiments, were carried out with different concentrations of gB2 and gB2ΔTM to determine the equilibrium binding levels in resonance units (RU). Correction for a small loss of binding capacity, approximately 30 RU, between repetitive injection cycles was made. Scatchard analyses from these data were performed by the method of Karlsson et al. (16). Equilibrium values (RU bound) from gB-binding experiments divided by the free concentration (in nanomoles) of gB in the flow cell were plotted against the gB bound at equilibrium (RU bound).

For measurement of gB2 or gB2ΔTM dissociation from heparin, several replicate experiments were carried out in which protein bound to the heparin surface was allowed to dissociate while the HBS solution traversed the flow cell at an increased rate (100 μl/min). These data permitted calculations of the rate constant for dissociation (k_{off}). Curve fitting of the dissociation curves was carried out with the IGOR Pro software (Wavemetrics Inc.).

To measure the ability of various glycosaminoglycans to competitively inhibit the gB2 binding to immobilized heparin, soluble glycosaminoglycans were premixed with gB2 before injection into the biosensor cell. The competitors included heparin (average molecular weight, 16,000), heparan sulfate from bovine kidney (average molecular weight estimated at 24,000), heparan sulfate from bovine intestine (average molecular weight, 7,500), dermatan sulfate from bovine mucosa (average molecular weight, 36,000), chondroitin sulfate A from bovine trachea (average molecular weight, 45,000) (all from Sigma Chemical), and completely desulfated N-acetylated heparin (Seikagaku). gB2 in the presence of each inhibitor was applied to the BIAcore flow cell, and binding was measured as the response above baseline.

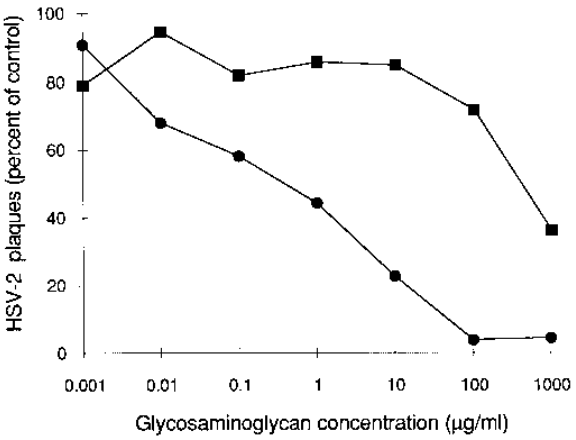

FIG. 1. Inhibition of HSV-2 plaque formation by glycosaminoglycans. HSV-2 strain 333 mixed with heparin (circles) or chondroitin sulfate A (squares) was used to inoculate monolayers. The mean of duplicate determinations is shown.

RESULTS

Glycosaminoglycan inhibition of HSV-2 infectivity. HSV-1 plaque formation was previously shown to be inhibited by heparin, but not by chondroitin sulfate A (21). HSV-2 plaque formation is sensitive to heparin (31) but is less sensitive to polylysine and neomycin than is HSV-1 plaque formation (17, 18), indicating a difference between the two HSV types in their responses to some charged inhibitors. Therefore, it was not obvious a priori how the infectivity of HSV-2 would be influenced by chondroitin sulfate A. The concentrations of heparin and chondroitin sulfate A necessary for the inhibition of plaque formation of HSV-2 in Vero cells were determined to compare the relative growth-inhibitory potencies of these two glycosaminoglycans (Fig. 1). In these experiments, heparin or chondroitin sulfate A was premixed with HSV-2 and used to inoculate Vero monolayers. The effective dose of heparin for 50% inhibition of plaque formation (ED_{50}) was less than 0.5 μg/ml (calculated at 0.4 μg/ml for this experiment), while the ED_{50} of chondroitin sulfate A was greater than 400 μg/ml (418 μg/ml for this experiment). From this and several other experiments we found that heparin inhibited HSV-2 infections at nearly 3 orders of magnitude lower concentration than did chondroitin sulfate A. These results indicate that, as with HSV-1, HSV-2 interaction with heparin is highly specific.

Binding of gB2 to the cell surface. The binding of gB2 to heparin-like cell surface glycosaminoglycans may play an important early role in HSV-2 infections. We examined the in-

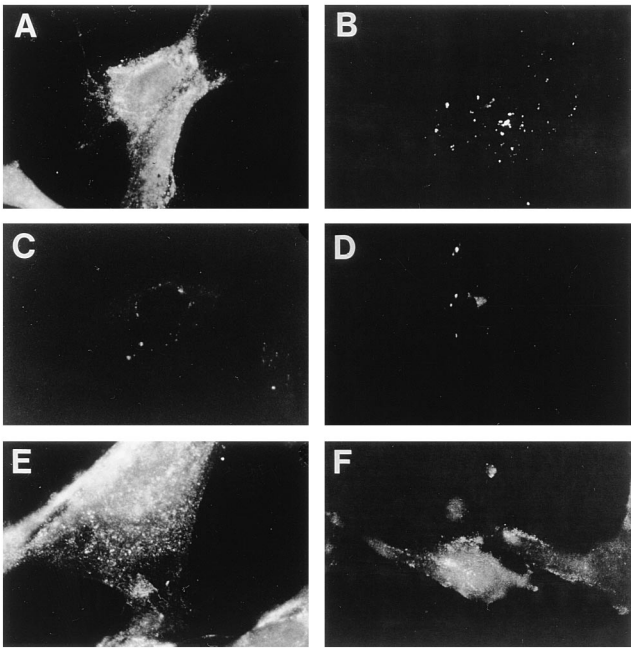

FIG. 2. Binding of gB2 to cell surfaces of live MRC-5 fibroblasts and competitive inhibition by glycosaminoglycans. (A and B) MRC-5 cells were incubated in soluble gB2 (A) or in buffer alone (B) and then fixed and labeled with rabbit polyclonal anti-HSV-2 antibody and fluorescein-conjugated anti-rabbit IgG. (C and D) gB2 binding was eliminated by preincubation with heparin at 100 μg/ml, and only residual perinuclear autofluorescence was observed. (E and F) gB2 binding was not reduced by preincubation with the same concentration of chondroitin sulfate A.

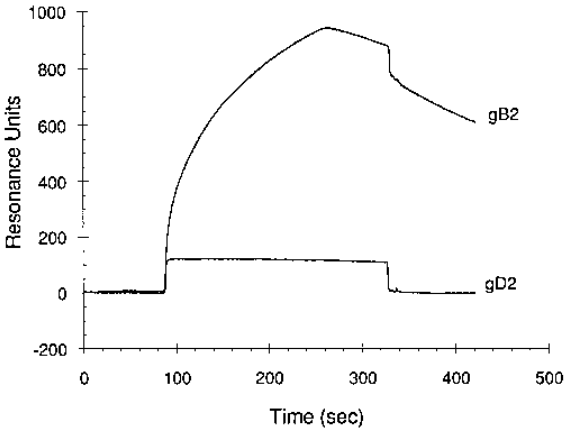

FIG. 3. Comparative binding of HSV-2 gB2 and gD2 to the heparin biosensor. gB2 (20 μg/ml) was injected at 4 μl/min onto the biotin-heparin surface. Binding of gB2 is shown in RU versus time in seconds with the baseline subtracted. gD2 was injected at the same concentration but failed to bind measurably to the heparin surface.

teraction of soluble recombinant gB2 with live MRC-5 cells to determine the glycosaminoglycan specificity of its binding to the cell surface. Monolayers of MRC-5 cells were incubated with gB2 at 4°C in the presence of azide to prevent internalization. The bound gB2 was visualized by immunofluorescent-antibody labeling. gB2 binding was seen as an intense speckled labeling of the entire cell surface (Fig. 2A). Control slides incubated without gB2 showed only background levels of fluorescence (Fig. 2B). Vero cells showed a similar intense cell surface pattern for gB2 binding, although the background fluorescence was higher (data not shown). When the gB2 was premixed with heparin (100 μg/ml), its binding to the MRC-5 cell surface was eliminated (Fig. 2C and D). In contrast, gB2 that had been premixed with the same concentration of chondroitin sulfate A labeled the MRC-5 cells in a pattern (Fig. 2E and F) that was indistinguishable from that observed with gB2 alone.

Previous studies by others showed that soluble gD2 inhibited HSV-1 and HSV-2 and that soluble gB2 failed to inhibit HSV-1 entry (14). We tested HSV-2 for plaque formation on cells with soluble gB2, gB2ΔTM, or gD2 added prior to and during adsorption and entry of the virus. gD2 inhibited just over 50% of HSV-2 plaques at 100 μg/ml. gB2 and gB2ΔTM at 100 μg/ml did not significantly inhibit HSV-2 plaques (data not shown), indicating that HSV-2 behaved similarly to HSV-1 in response to preincubation of cells with soluble gD2 and gB2 proteins. The apparent high density of heparin-like gB2 receptors on the cell surface as seen in Fig. 2 may be one reason that HSV-2 adsorption was not blocked by the soluble gB glycoproteins.

Plasmon resonance measurement of gB2 binding. We used a biosensor system to analyze further the interaction of gB2 with glycosaminoglycans. A biosensor with a surface of immobilized heparin was prepared and tested for binding of gB2 or of gD2. gB2 applied at 20 μg/ml bound readily to the heparin surface, as shown in the sensorgram in Fig. 3.

The biotin-heparin was unaffected in ability to block HSV-2 plaque formation, since both heparin and the biotin-heparin had the same ED_{50} for HSV-2 plaque inhibition (data not shown). As is typical of biosensor data, the gB2 sensorgram can be divided into several phases. From 0 s (at injection) to approximately 87 s, the RU remained at baseline while residual buffer in the system transited the flow cell. During a second phase, which lasted several seconds, a sharp upward rise of RU was recorded due to the change in refractive index of the flow cell solution because of the concentration of gB2 in it. An association phase for gB2 binding to the biotin-heparin surface then took place over the next few minutes, until a maximum of 944 RU was observed. At about 325 s into the experiment, the gB2 solution was replaced by buffer alone in the flow cell, resulting in an immediate drop in RU (due to the refractive index of the buffer) followed by a slow dissociation phase.

A very different plasmon resonance sensorgram was observed when the gD2 protein was applied to the heparin biosensor at the same concentration (Fig. 3). After an initial rise of 123 RU, due to the increased refraction by the protein-containing buffer, no additional rise in RU was seen, indicating that gD2 did not bind to immobilized heparin. Thus, gB2 binds specifically to immobilized heparin, while gD2 does not.

We next compared the binding of gB2 to a heparin surface, containing 222 RU of immobilized heparin, with the binding of gB2 to a surface of biotinylated chondroitin sulfate A, containing 307 RU of immobilized chondroitin sulfate A, or to a control surface of streptavidin-dextran alone. Equal concentrations of gB2 (10 μg/ml) yielded 541 net RU of gB2 binding to the heparin surface as compared to 58 net RU of gB2 binding to a surface of chondroitin sulfate A (Fig. 4). A net gB2 binding of less than 5 RU was observed when gB2 was applied to the control flow cell surface of streptavidin-dextran alone in Fig. 4. These results show that gB2 interacts more readily with immobilized heparin than with the immobilized chondroitin sulfate A.

gB2 binding kinetics. To calculate the kinetics of binding of gB2 to heparin, twofold increasing concentrations of recombinant gB2 ranging from 31 nM (3 μg/ml) to 1,000 nM (100

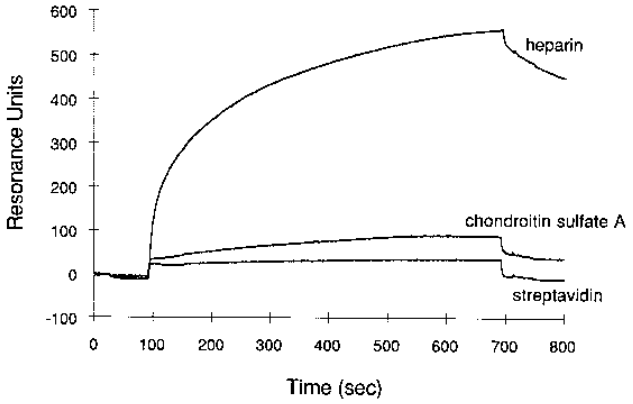

FIG. 4. Binding of gB2 to heparin, chondroitin sulfate A, or control streptavidin-dextran biosensor surfaces.

μg/ml) were injected into the immobilized heparin flow cell (Fig. 5A). Each curve shows the results for a single gB2 concentration. A higher equilibrium binding value was observed with each successively higher gB2 concentration. The equilibrium binding values, in RU, for each of the concentrations were plotted for Scatchard analysis to determine the affinity of binding. An affinity constant (K_d) of 7.7×10^{-7} M was calculated from the slope of this line (Fig. 6).

Because the gB2 tested represents the ectodomain of only this protein, a second recombinant construct, the gB2ΔTM protein, was tested for binding to immobilized heparin. This protein consists of the gB2 ectodomain of HSV-2 gB2 joined to its cytoplasmic domain but lacking the transmembrane domain. It is therefore also soluble. Scatchard analysis showed that the gB2ΔTM also bound heparin with a similar but slightly lower affinity ($K_d = 9.9 \times 10^{-7}$ M [Fig. 6]).

The rates for the dissociation (k_{off}) of gB2 and gB2ΔTM from the heparin surface were determined from the dissociation curves of duplicate experiments. The biosensor data plotted in Fig. 7 were modeled by nonlinear curve fitting for the 1,000 s from the beginning of the dissociation. These results show that both gB2 and gB2ΔTM interact with immobilized heparin in a reversible manner. We calculated a rate of dissociation (k_{off}) for gB2 of 4×10^{-4} s^{-1}. The dissociation of

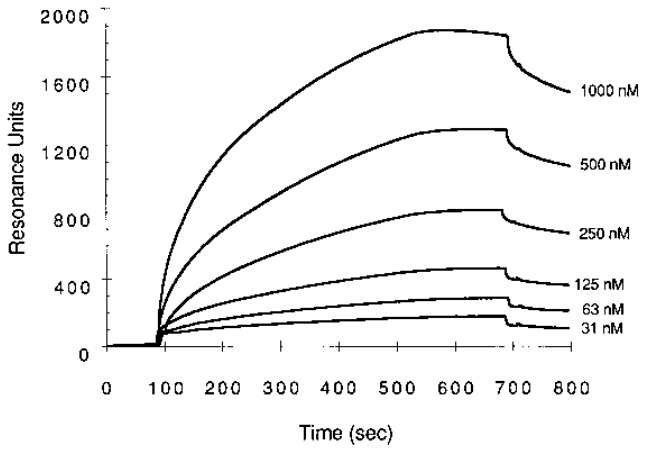

FIG. 5. gB2 at concentrations ranging from 31 to 1,000 nM was injected onto the heparin-biotin biosensor chip at 2 μl/min. gB2 reached a new equilibrium value with each higher concentration. The resulting curves from multiple runs, with baselines subtracted, were overlaid for comparison.

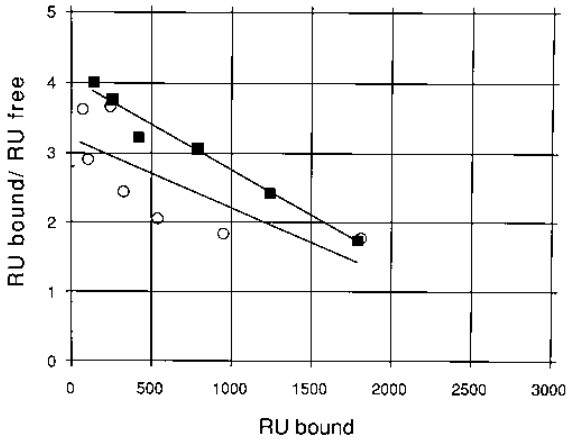

FIG. 6. Scatchard analyses of binding of recombinant gB2 and gB2ΔTM to immobilized heparin. Equilibrium binding levels (RU bound) for gB2 (solid squares) from Fig. 3 were plotted on the *x* axis against RU observed at equilibrium, divided by the continuously replenished gB2 concentration (RU free) on the *y* axis. gB2ΔTM binding (open circles) was analyzed similarly. Affinity constants (K_ds) of 7.7×10^{-7} and 9.9×10^{-7} M were calculated for gB2 and gB2ΔTM, respectively.

gB2ΔTM (Fig. 7) was slightly more rapid ($k_{off} = 1.1 \times 10^{-3}$ s^{-1}), contributing to the lower observed affinity constants for gB2ΔTM than for gB2.

Structural determinants of gB2-glycosaminoglycan binding. Further evidence regarding the relative specificity of gB2 binding to heparin was gained by adding soluble glycosaminoglycan competitors to inhibit its interaction with immobilized heparin. Soluble heparin in increasing concentrations was premixed with gB2 before interaction with the immobilized heparin in the flow cell. The curves plotted in Fig. 8A show a dose response of soluble heparin inhibition of gB2 binding to immobilized heparin: as little as 0.1 μg of soluble heparin per ml inhibited gB2 binding substantially, while 1 μg/ml eliminated most gB2 binding and higher concentrations eliminated all detectable gB2 binding. Chondroitin sulfate A inhibition of gB2 binding to immobilized heparin was similarly determined,

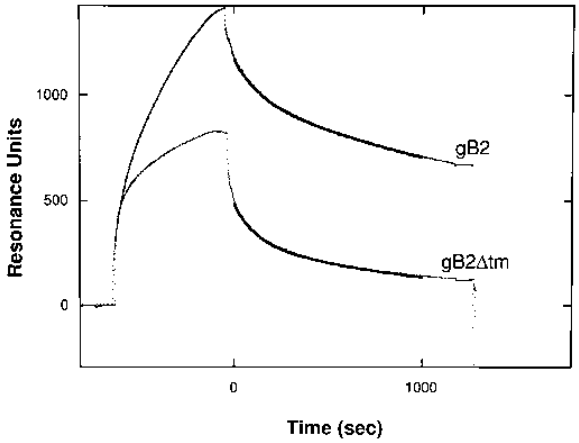

FIG. 7. The dissociation rate constants (k_{off}) of gB2 (A) and gB2ΔTM (B) from immobilized heparin were determined by binding the glycoproteins to heparin at a high level and then replacing the gB-containing buffer with buffer alone at a high flow rate (100 μl/min). The biosensor data were modeled by nonlinear curve fitting of data points from 0 s, at the beginning of the dissociation phase to 1,000 s.

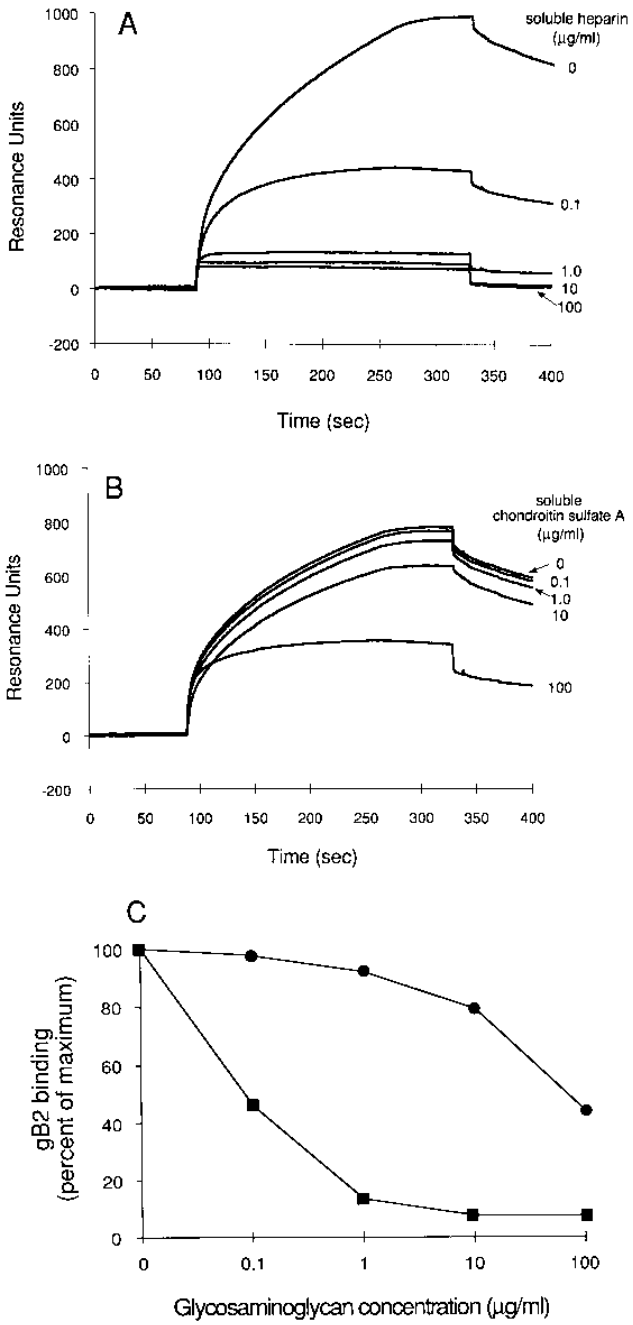

FIG. 8. Competitive inhibition of gB2 binding to the heparin biosensor surface by soluble heparin or chondroitin sulfate A. (A and B) Soluble heparin at 0 to 100 μg/ml (A) or soluble chondroitin sulfate A at 0 to 100 μg/ml (B) was mixed with gB2 before application to the heparin surface. (C) Inhibition by heparin (squares) or chondroitin sulfate A (circles) was plotted as the percentage of gB2 maximal binding where no inhibitor was present.

revealing that much higher concentrations are needed for comparable levels of inhibition (Fig. 8B).

To estimate the concentration of each soluble glycosaminoglycan required for 50% inhibition (ED_{50}) of gB2 binding to immobilized heparin, the maximal values from sensorgrams in Fig. 8A and B were plotted as percentages of control values, where no inhibitor was present, versus the log concentration of the glycosaminoglycans in Fig. 8C. The calculated ED_{50} for heparin was 0.08 μg/ml, while the ED_{50} for chondroitin sulfate A was nearly 3 orders of magnitude higher at 65 μg/ml. These results show that soluble heparin is far more effective a competitor of gB2 binding to immobilized heparin than is chondroitin sulfate A.

TABLE 1. Inhibition of HSV-2 gB2 binding to the biotin-heparin biosensor chip by soluble glycosaminoglycans

Glycosaminoglycan	ED_{50} (μg/ml)
Heparin	0.08
Heparan sulfate (bovine intestine)	1
Dermatan sulfate	4
Heparan sulfate (bovine kidney)	5
Chondroitin sulfate A	65
Desulfated, N-acetylated heparin	>2,000

Heparan sulfate and dermatan sulfate glycosaminoglycans may represent functional alternative receptors for HSV-1 (1, 31). Inhibition of gB2 binding by heparan sulfate from bovine intestinal mucosa or bovine kidney and by dermatan sulfate were tested with the biotin-heparin chip of the biosensor. Heparan sulfate (bovine intestine) inhibited gB2 binding at relatively low levels (ED_{50} = 1 μg/ml). The ED_{50}s of heparan sulfate (bovine kidney) or dermatan sulfate were higher but were still much less than that calculated for chondroitin sulfate A (Table 1). Our results show that both heparin-like (heparin and heparan sulfate) and dermatan sulfate glycosaminoglycans can interact with HSV-2 gB.

The degree of sulfation is important to the antiviral effect of heparin (10), which suggests that sulfation may be an important determinant of the interaction of gB2 with glycosaminoglycans as well. To address this possibility, heparin that had been completely desulfated and N-acetylated was tested for inhibition of gB2 binding to immobilized heparin. Desulfated heparin failed to inhibit gB2 binding to heparin (Table 1). Thus, the sulfation of heparin is important to its recognition by gB2.

DISCUSSION

Using a variety of methods, including quantitation of intermolecular binding reactions in real time, we showed that HSV-2 gB2 binds to glycosaminoglycans with high specificity. The existence of heparin-like moieties on the cell surface, the inhibition of gB2 binding to the cell surface and plaque formation by soluble heparin, and the fact that gB2 is an essential protein all suggest that the replication of HSV-2 begins with an interaction between gB and cell surface glycosaminoglycans of the heparan sulfate type.

We found that the glycosaminoglycan-binding site(s) of HSV-2 gB also interacts with dermatan sulfate. The presence of iduronic acid residues in the glycosaminoglycan disaccharide repeat appears to be important to interaction with recombinant gB2, since heparin, heparan sulfate, and dermatan sulfate all contain α-L-iduronic acid (13). Chondroitin sulfate A, which bound gB2 poorly, is a weak competitive inhibitor of gB2 binding to biotin-heparin and failed to inhibit gB2 binding to MRC-5 human fibroblasts, lacks iduronic acid, and differs from dermatan sulfate only at this residue. A related finding by others, i.e., that dermatan sulfate strongly inhibited HSV-1 plaque formation in cells unable to synthesize heparan sulfate (1), raises the possibility that dermatan sulfate proteoglycans are alternative initial receptors for both HSV-1 and HSV-2.

The degree of sulfation of the glycosaminoglycan is also an important determinant for recognition by gB2. Heparin is

more highly sulfated than heparan sulfate and was a better competitive inhibitor of gB2 binding to immobilized heparin. Desulfated, N-acetylated heparin was unable to compete for gB2 binding. For other heparin-binding proteins, clusters of basic amino acids are important to heparin binding (3). It is thought that these basic amino acid clusters initiate heparin binding through interaction with the negatively charged sulfate groups. Although the heparin-binding site(s) of gB (of HSV-1 or HSV-2) have not been identified, clusters of basic amino acids previously noted in the N terminus of HSV-1 gB and proposed to represent heparin-binding sites (26) are partially conserved in the gB2 sequence.

The external domain of HSV-2 gB bound heparin with a K_d calculated at 7.7×10^{-7} M. Purified soluble gB from bovine herpesvirus type 1 binds to live cells with a K_d of 5×10^{-7} M (20), a remarkably similar binding constant to that which we have found for the interaction of gB2 with the biotin-heparin biosensor technology. The similarity of observations indicates that the affinity of heparin binding by gB has been conserved in these two related alphaherpesviruses.

In our experiments, the formation of the gB2-glycosaminoglycan complex was reversible. The release of gB from heparin-like glycosaminoglycans may be advantageous to HSV-2 under certain circumstances when the virion contacts heparin-like glycosaminoglycans in the extracellular space, in basement membranes, or on inappropriate cell targets. It may also be important that gB2 be able to dissociate from heparin-like glycosaminoglycan to allow the release of newly made virus from the glycosaminoglycans on the surface. gB2 of HSV-2 may have evolved an affinity for heparin-like receptors that is sufficiently strong to promote cellular attachment but still weak enough to allow release of the virion if subsequent glycoprotein interactions necessary for virus penetration do not occur within a given time frame.

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