JOURNAL OF VIROLOGY, May 1981, p. 564-570
0022-538X/81/050564-07$02.00/0
Vol. 38, No. 2

Unusual Lectin-Binding Properties of a Herpes Simplex Virus Type 1-Specific Glycoprotein

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Received 14 July 1980/Accepted 11 February 1981

Lysates from herpes simplex virus type 1-infected cells were subjected to affinity chromatography on soybean and *Helix pomatia* lectins. One of the virus-specified glycoproteins, probably the herpes simplex virus type 1-specific gC glycoprotein, bound to the lectins and was eluted with *N*-acetylgalactosamine. The affinity chromatography permitted a high degree of purification of the type-specific glycoprotein with respect to both host cell components and other viral glycoproteins. The lectin affinity pattern of this glycoprotein indicates the presence of a terminal α-*N*-acetylgalactosamine in an oligosaccharide, a finding not reported previously for glycoproteins of enveloped viruses.

Five glycoproteins specified by the herpes simplex virus type 1 (HSV-1) genome are demonstrable in the viral envelope and the plasma membrane of HSV-infected permissive cells (2, 29). One of the glycoproteins, designated gB, is involved in HSV-induced cell fusion, formation of polykaryocytes, and mechanisms behind HSV penetration (16, 26). Glycoprotein gC, carrying HSV type specificity, also seems of importance in the process of HSV-induced cell fusion. Another glycoprotein, gE, has been identified as the HSV-induced Fc receptor of infected cells (2).

Recently, we observed that at least one or more of the HSV-specified glycoproteins, in contrast to glycoproteins of other enveloped viruses studied, might contain alkali-sensitive oligosaccharide chains, presumably with an *O*-glycosidic type of linkage between the saccharide and peptide (S. Olofsson, J. Blomberg, and E. Lycke, submitted for publication). The general composition of oligosaccharides of this class differs from that of the *N*-glycosidic type observed in viral glycoproteins (11, 12, 18, 23, 25, 27). We have subjected HSV-specific glycoproteins to affinity chromatography on lectins (soybean lectin and *Helix pomatia* lectin) known to bind *N*-acetylgalactosamine (GalNAc) residues, which often occur in oligosaccharides *O*-glycosidically linked to peptides of membrane glycoproteins (4, 10, 12).

The present report demonstrates that the HSV-1-specific glycoprotein (gC) can be extensively purified by means of *H. pomatia* or soybean lectin chromatography and that the affinity pattern encountered is consistent with the presence of a terminal α-GalNAc in the oligosaccharide.

(A preliminary report was presented in October, 1980 at the Seventh Scandinavian Virus Symposium in Turku, Finland.)

MATERIALS AND METHODS

Viruses and cells. HSV-1 strain F was used for production of HSV. In addition, vesicular stomatitis virus (VSV) was used in control experiments. The viruses were cultivated at 36.5°C in green monkey kidney cells (GMK AH-1) and baby hamster kidney cells (BHK-21). Cells were grown in Eagle minimal essential medium supplemented with 100 IU of penicillin per ml, 100 μg of streptomycin per ml, and 10% fetal calf serum. For maintenance, the same medium without calf serum was used.

Chemicals and radiochemicals. D-[1-^{3}H]glucosamine (5 Ci/mmol) was purchased from the Radiochemical Centre, Amersham, England. Soybean lectin, *H. pomatia* lectin-Sepharose 6MB, and wheat germ lectin-Sepharose 6MB were purchased from Pharmacia Fine Chemicals, Uppsala, Sweden. A 10-mg amount of soybean lectin was coupled to 0.5 g of freeze-dried Affigel 10 (Bio-Rad Laboratories, Richmond, Calif.) and reconstituted in 12.5 ml of a 0.1 M phosphate buffer, pH 7.4. Tris was obtained from Merck & Co., Stockholm, Sweden.

Preparation of infected cells. GMK AH-1 cells in 5-cm petri dish cultures were infected with HSV-1 by using a multiplicity of 10 to 20 PFU per cell. After 1 h of adsorption, the fluid medium was replaced by maintenance medium, and at 4 h postinfection, 50 μCi of glucosamine label per ml was added. Acid hydrolysis and subsequent thin-layer chromatography revealed that there was no conversion of glucosamine label into amino acids. At 16 h postinfection the cells were harvested by scraping and pelleted by low-speed centrifugation. The pellet was solubilized in 1 ml of 1% Triton X-100 in Tris-buffered saline (0.15 M NaCl, 0.02 M Tris-hydrochloride, pH 7.5). The resulting mixture was subjected to centrifugation for 60 min at 100,000 $\times$ g, and the supernatant was studied by

affinity chromatography. For large-scale production (Fig. 1), HSV-1 was inoculated onto 1,000-ml roller bottle cultures of BHK-21 cells at a multiplicity of 10 PFU per cell. At 5 h postinfection, the medium was discarded, and the cells were washed twice in maintenance medium. A 50-ml amount of maintenance medium was added, and the cultures were incubated until complete cytopathic effect was developed. The cells were then shaken off the glass and separated by centrifugation at 1,500 × *g* for 15 min. The packed cells were stored at −70°C.

Crude preparations of membranes of HSV-infected cells were produced by the method of Springer et al. (30). A 10-ml amount of packed frozen cells was thawed and placed on ice. The volume was adjusted to 40 ml with 0.025 M Tris-hydrochloride, pH 8.0, and the cell suspension was incubated on ice for 10 min. The cells were treated with three strokes in a tight-fitting Dounce homogenizer and centrifuged at 1,500 × *g* for 15 min at 4°C. The supernatant was decanted, and again the pellet was suspended, treated in the homogenizer, and centrifuged. Two more extracts were thus decanted. The three supernatants were pooled and centrifuged at 27,000 × *g* for 1 h. The membranes remaining in the supernatant were pelleted at 160,000 × *g* for 1 h. A 4-ml amount of pelleted membranes was suspended in 32 ml of cold 0.1 M glycine-NaOH buffer, pH 8.8, and centrifuged at 160,000 × *g* for 1 h. Finally, the washed membranes were suspended in 32 ml of glycine-NaOH buffer containing 1% Triton X-100 (wt/vol). The suspension was homogenized by 40 strokes in the homogenizer, incubated on ice for 10 min, and centrifuged at 100,000 × *g* for 1 h. The supernatant was used for affinity chromatography.

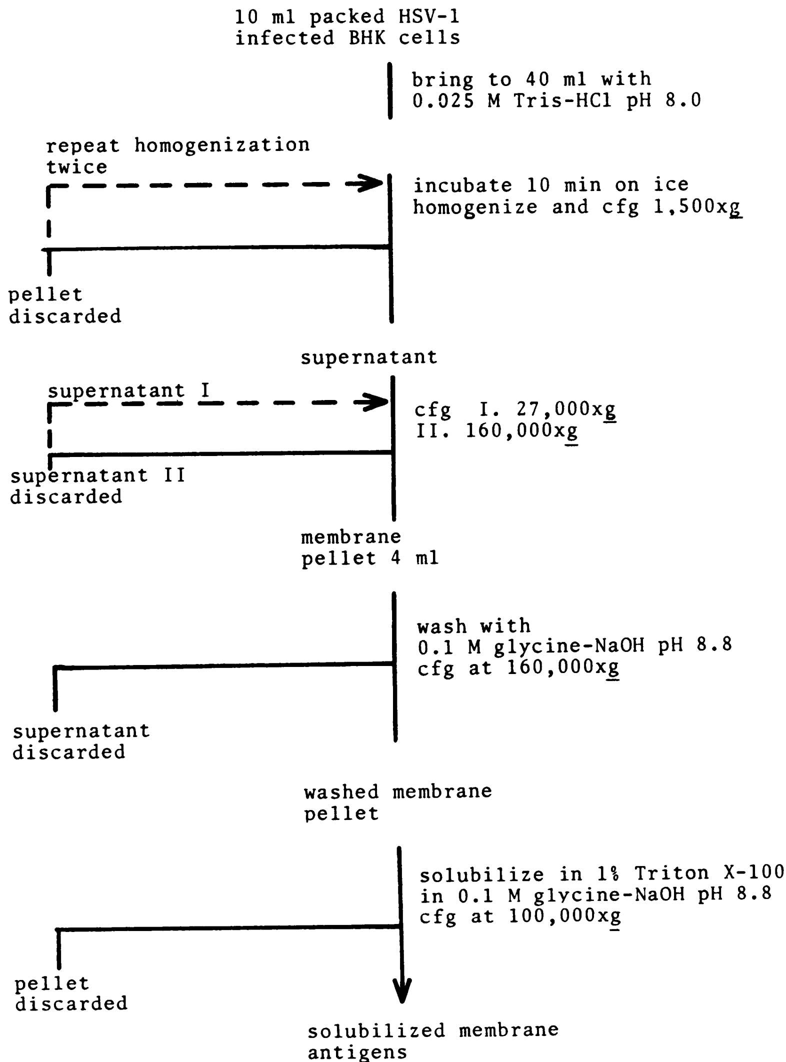

FIG. 1. *Flow sheet for preparation of membrane Ag's from HSV-1-infected BHK-21 cells. cfg, Centrifugation.*

Affinity chromatography. Two types of columns for gel-bound lectin affinity chromatography were used. The preparations of GMK AH-1 cells were analyzed on gel-bound lectins in columns made of standard-sized Pasteur pipettes (inner diameter, 6 mm). A 1-ml amount of gel-bound lectin yielded a bed height of 9 mm. The Triton X-100–solubilized material (0.4 ml) was added to the column and was allowed to adsorb for 15 min at room temperature. The gel was washed with 10 bed volumes of 1% Triton X-100 in Tris-buffered saline. The flow rate did not exceed 15 drops per min. If not otherwise stated, the biospecifically bound material was eluted by adding 0.01 M solutions of GalNAc, *N*-acetylglucosamine (GlcNAc), or galactose in Tris-buffered saline with 1% Triton X-100.

Fractions, each with a volume of 0.5 ml, were collected and analyzed for radioactivity by liquid scintillation. To the fraction which contained an adequate amount of radioactivity, trichloroacetic acid was added (10%). Pellets after centrifugation were dissolved in the electrophoresis sample buffer (0.0625 M Tris, 2% sodium dodecyl sulfate, 10% glycerol, 5% 2-mercaptoethanol, 0.001% bromophenol blue, pH 6.8) and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis in 7.5% gel slabs as described by Laemmli (13). Heavy chains of immunoglobulin G (IgG) (molecular weight, 50,000) bovine serum albumin (68,000), and β-galactosidase (130,000) were used as molecular weight markers. The gels were then treated with an autoradiography enhancer solution (Enhance, New England Nuclear Corp., Dreieich, West Germany), dried under vacuum, and autoradiographed on Kodak X-Omat film.

The Triton X-100–solubilized antigens from BHK-21 cells were applied on columns (diameter, 16 mm) containing 8 ml of *H. pomatia* lectin coupled to Sepharose 6MB. The gel was equilibrated with 0.02 M Tris-buffered saline and 0.5% Triton X-100 and was developed at 4 ml/h with the same buffer. The biospecifically bound substances were eluted with 0.01 M GalNAc.

HSV antigen (Ag)-containing fractions were identified by counterimmunoelectrophoresis (5) with hyperimmune antisera (rabbit) to HSV-1 and HSV-2 and an HSV-1–specific antiserum, prepared by adsorption with glutaraldehyde-polymerized HSV-2 Ag as previously described (6). For production of hyperimmune antisera to HSV, five rabbits were inoculated into the scarified skin of the trunk bilaterally. One month after the primary infection, the rabbits were inoculated in the eye and developed keratitis. Two weeks after healing of the corneal lesions, the animals were bled, sera were tested for precipitins to HSV Ag's, and the most potent sera were selected for use. In addition, monospecific antisera to Ag-11 (gA and gB), Ag-6 (gC), and Ag-8 (gD) prepared by immunization of rabbits with single immunoprecipitates from crossed immunoelectrophoresis were kindly supplied by B. F. Vestergaard, Copenhagen, Denmark. The antiserum to Ag-6 reacts only with glycoprotein gC and its precursors as determined by crossed immunoelectrophoresis and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (19).

Protein concentrations were determined by the method of Lowry et al. (15), modified for measurement in Triton X-100–containing solutions (3).

RESULTS

HSV glycoprotein-binding properties of lectins with affinities for GalNAc. Glucosamine-labeled glycoproteins from Triton X-100 lysates of HSV-infected and, as a control, VSV-infected GMK AH-1 cells were subjected to soybean lectin chromatography. After adsorption, the gels were washed and eluted with 0.01 M GalNAc.

The chromatograms (Fig. 2) demonstrated that the VSV material passed through the column without demonstrable affinity by soybean lectin. This result was expected by the reported structure of the oligosaccharide of the VSV glycoprotein (18). More than 10% of labeled HSV material adsorbed to the lectin and was eluted in a distinct peak. Small, but significant, amounts of labeled host cell glycoproteins were adsorbed by the lectin. However, the host cell glycoproteins which specifically interacted with the lectin amounted to approximately 10% of the amount of lectin-adsorbed glycoproteins of HSV-infected cells. Both nonadsorbed and lectin-adsorbed and eluted HSV materials were studied with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Fig. 3). With the nonadsorbed material, several glycosylated species were observed in the region corresponding to molecular weights ranging from 50,000 to 130,000. With the lectin-adsorbed and eluted material, only one glucosamine-labeled band was detected.

Table 1 shows the results of several affinity chromatography experiments. Preparations derived from both HSV- and VSV-infected GMK AH-1 and BHK-21 cells were studied. In addition to the two different types of GalNAc-bind-

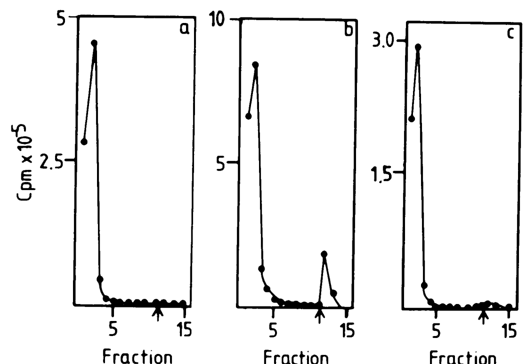

FIG. 2. *Soybean lectin chromatography of [^{3}H]-glucosamine-labeled preparations of VSV-infected (a), HSV-infected (b), and uninfected (c) cells. Arrows show positions of addition of 0.01 M GalNAc.*

ing lectins used, chromatography with wheat germ lectin with affinity for GlcNAc residues was included as an additional control. About 5 to 12% of glucosamine-labeled materials of both types of HSV-infected cells were specifically bound to the soybean and *H. pomatia* lectins. The fraction of lectin-bound material of preparations of uninfected cells was at least five times less (0.05 to 1.1%). In no experiment did material from VSV-infected cells adsorb to the *H. pomatia* or soybean lectins. On the other hand, the wheat germ, as expected, adsorbed glucosamine-labeled material of HSV- and VSV-infected cells almost equally well (5.1 to 8.1%).

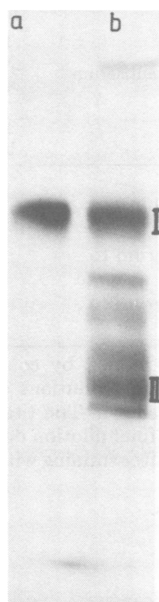

FIG. 3. *Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of fractions obtained from soybean lectin of [³H]glucosamine-labeled materials of HSV-infected cells. Material eluting after addition of 0.01 M GalNAc corresponded to fractions 12 to 14 of Fig. 2. (a) Material washed out of the column, corresponding to fractions of 1 to 4 of Fig. 2. (b) I and II indicate positions of glycoproteins gA, gB, and gC (I) and gD (II).*

The specificity of the binding to the lectins is shown in Table 2. The labeled glycoprotein was most efficiently eluted from both of the lectins with GalNAc. GlcNAc was more effective than galactose for the elution of *H. pomatia* lectin-bound glycoprotein, whereas galactose, but not GlcNAc, eluted the soybean lectin-bound material. This pattern was anticipated by the reported specificities of the lectins studied (4, 10, 14).

The specific affinity of HSV glycoproteins for the GalNAc-binding lectins thus demonstrated was used for purification purposes. Table 3 shows contents of protein and HSV Ag titers before and after separation of the glycoprotein. An anti-Ag-6 serum (an HSV-1-specific antiserum) was used. The specific activity, i.e., the relative concentration of the HSV Ag titer to the protein concentration, was increased from 6 to 46 by adsorption to *H. pomatia* lectin and subsequent elution with 0.01 M GalNAc. Crude preparations of membranes of HSV-infected cells produced four precipitation lines with HSV-1 antiserum in counterimmunoelectrophoresis (Fig. 4). Three of these lines were demonstrable with material which did not interact with the *H. pomatia* or soybean lectins, whereas material that revealed binding affinity and eluted from the column with GalNAc produced one line only.

Immunological characterization of HSV glycoprotein preparations purified by lectin affinity chromatography. In a series of

TABLE 2. *Binding of radiolabeled HSV glycoprotein to H. pomatia and soybean lectins*

Lectin	mM solution of following eluting sugar necessary to release 50% of label[a]		
	Galactose	GlcNAc	GalNAc
H. pomatia	500	50	<0.5
Soybean	5	>500	1

[a] A total of 10,000 cpm of glucosamine label was bound to the lectins. Elution was performed stepwise with 0.5, 1, 5, 10, 50, 100, and 500 mM solutions of the sugars.

TABLE 1. *Lectin affinity chromatography of HSV and VSV glycoproteins*

Lectin (eluting sugar)	% Label bound to lectin and eluted in following cells:					
	GMK AH-1			BHK-21		
	Uninfected	VSV infected	HSV infected	Uninfected	VSV infected	HSV infected
Soybean (GalNAc)	0.9 (580)[a]	≤0.5 (789)	12.1 (1.852)	≤0.5 (231)	≤0.5 (62)	4.9 (537)
H. pomatia (GalNAc)	1.0 (546)	≤0.5 (62)	5.6 (62)	1.1 (113)	≤0.5 (36)	10.8 (285)
Wheat germ (GlcNAc)	30.7 (260)	5.1 (33)	8.1 (452)			

[a] Counts per minute × 10^{-3} added to columns are shown within parentheses.

counterimmunoelectrophoresis studies, glycoprotein preparations purified by soybean or *H. pomatia* lectins were studied (Fig. 4 and Table 4). The following potent and well-characterized antisera (5, 6, 20, 21) were used: an HSV-1 rabbit antiserum, an HSV-1 rabbit antiserum adsorbed with HSV-2 Ag, anti-Ag-6, anti-Ag-8, and anti-Ag-11 (HSV-1) rabbit sera (from B. F. Vestergaard), and a hyperimmune serum to HSV-2.

In Table 4, results of precipitation of *H. pomatia* lectin-purified HSV glycoproteins with HSV antisera are shown. The titers of precipitating Ag's against these antisera clearly demonstrated the enrichment of an Ag corresponding to Ag-6 (gC). None of Ag's corresponding to Ag-8 (gD) and Ag-11 (gA and gB) were demonstrated in the *H. pomatia*-purified material. The 16-fold (at least) reduction in Ag activity corresponding to Ag-11 (gA and gB) should be emphasized since gA, gB, and gC are electrophoretically relatively closely associated. In the double-diffusion gel, one single precipitation line was obtained when the lectin-purified material was tested against hyperimmune antiserum to HSV-

TABLE 3. *Affinity chromatography (H. pomatia lectin) as a preparatory step for purification of HSV glycoprotein*

Preparatory step	Total protein (mg)	Protein concn (mg/ml)	HSV Ag (titer)[a]	Sp act (titer/mg)[b]
Supernatant after centrifugation at 100,000 × *g* of detergent-solubilized membranes	49	2.72	16	6
Adsorption of *H. pomatia* lectin and elution with GalNAc	2.8	0.35	16	46

[a] Titers were determined by testing fourfold dilutions of HSV Ag's against anti-Ag-6 antiserum in counterimmunoelectrophoresis. Values represent reciprocals of the final dilutions demonstrating precipitates detectable after staining with Coomassie brilliant blue.

[b] Specific activity is expressed as the ratio of Ag titer to protein content determined by a modified Lowry test (3, 15).

TABLE 4. *Precipitating activity of H. pomatia-binding HSV Ag*

Specificity of HSV antiserum	Ag[a] titer	
	Triton X-100–solubilized membranes	Glycoproteins eluted from *H. pomatia* lectin
Ag-8 (gD)	4	<1
Ag-11 (gA + gB)	16	<1
Ag-6 (gC)	16	16
Hyperimmune antiserum to HSV-1	16[b]	16[c]
Hyperimmune antiserum to HSV-2	16	<1

[a] Titers were determined by counterimmunoelectrophoresis with fourfold dilutions of the HSV Ag's against the antisera shown. The titer is expressed as the reciprocal of the final dilution demonstrating precipitates detectable after staining with Coomassie brilliant blue.

[b] Four precipitates.

[c] One precipitate.

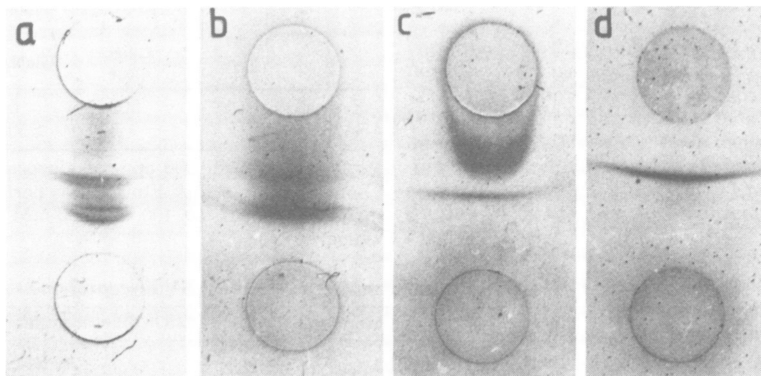

FIG. 4. *Precipitation pattern after countercurrent electrophoresis of Triton X-100-solubilized HSV-1 membrane-associated Ag's and fractions from affinity chromatography. Plates were stained with Coomassie brilliant blue. The a, b, and c fractions were tested against a regular HSV-1 hyperimmune serum (see the text). a, Triton X-100-solubilized Ag's (four precipitation lines); b, Ag's without affinity for H. pomatia lectin (three lines); c, Ag's eluted with GalNAc (one line); d, Ag's eluted with GalNAc, and the test was performed with monospecific rabbit anti-Ag-6 antibodies (one line).*

1. This precipitate formed a line of fusion with the precipitate developed between the lectin-purified material and the monospecific anti-Ag-6 (gC) serum (Fig. 5).

DISCUSSION

Glycoproteins of a number of enveloped viruses studied, including VSV, Sindbis, Semliki Forest, influenza, and several retroviruses, contain only oligosaccharides which have GlcNAc *N*-glycosidically linked to the polypeptide backbone (11, 18, 23, 25, 27). In contrast to the VSV glycoprotein, HSV-specified glycoproteins revealed, in addition, saccharide-peptide linkages sensitive to treatment with alkaline borohydride (Olofsson et al., submitted for publication). As the latter finding suggested the presence also of *O*-glycosidic oligosaccharides in HSV glycoproteins, a further characterization of the HSV glycoprotein structure by means of lectin affinity chromatography seemed pertinent.

HSV glycoproteins have been characterized by their binding to concanavalin A and wheat germ lectins (7, 24, 31). These lectins have affinity for internal α-mannose and GlcNAc, respectively, of oligosaccharides (1, 28), which, in general, are linked *N*-glycosidically to the polypeptide (12, 17). *H. pomatia* and soybean lectins which were used in the present study have specific affinities for terminal GalNAc residues, but some differences in the specificities of these two lectins are recognized. The *H. pomatia* lectin has affinity for α-D-GalNAc and, to some extent, α-D-GlcNAc (4, 9, 10, 14). Thus, the α-anomeric glycosidic linkage and the equatorial *N*-acetyl group at C-2 strongly contribute to binding properties of the *H. pomatia* lectin (4). The soybean lectin, on the other hand, has affinity for both α- and β-D-GalNAc, but the galactose epimeric configuration at C-4 is considered essential for its binding properties (4). Both lectins bind to terminal sugars (4, 10). Assuming that one and the same oligosaccharide structure was engaged in the binding to the two lectins, the lectin affinity of an HSV glycoprotein, gC, is consistent with α-D-GalNAc as a terminal sugar of the oligosaccharide. Our results (Table 2) are in agreement with this hypothesis. The results also support the assumption of the existence of oligosaccharides *O*-glycosidically linked to HSV glycoproteins since α-D-GalNAc exists as a terminal sugar in several types of oligosaccharides which are *O*-glycosidically linked to the polypeptide but is rare, if at all present, in *N*-glycosidic heterosaccharide-peptide structures (12, 17).

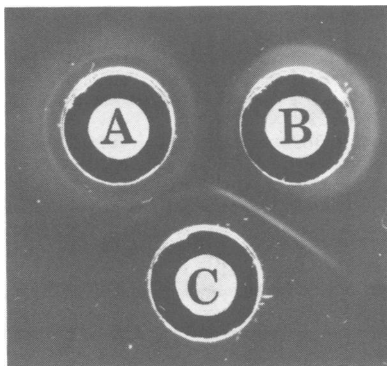

FIG. 5. *Double diffusion in gel with HSV-1 Ag purified by H. pomatia lectin chromatography. The agarose was dissolved in 0.025 M Tris-hydrochloride buffer, pH 8.0, with 0.2% (wt/vol) Triton X-100, and gels of 1% (wt/vol) were used. Samples of 10 μl of Ag and antisera were added, and the immunodiffusion was performed at room temperature for 2 days. The precipitates were photographed without previous staining. Hyperimmune antiserum (rabbit) to HSV-1 (A) and monospecific antiserum (rabbit) to Ag-6 (gC) (B) were tested against an HSV-1 Ag (C) that was adsorbed and eluted from H. pomatia lectin with 0.01 M GalNAc.*

The induction of structures with affinity for GalNAc-binding lectins in HSV-infected cells may result from different metabolic events. One possibility is HSV-induced alterations in the host cell glycosylation, including modification of cellular glycosyl transferases and activation of quiescent enzymes. Another is the synthesis of new virus-coded enzymes. In this context, it should be mentioned that we have observed HSV-induced changes in the kinetic properties of galactosyl and sialyl transferases in association with HSV infection of cells (22).

The *H. pomatia* and soybean lectins provide unique possibilities for enrichment and purification of HSV glycoprotein by affinity chromatography. Use of specific antisera against well-characterized HSV Ag's (19) suggested that, in this way, glycoprotein gC was enriched and purified.

Receptors for GalNAc-binding lectins are relatively sparsely distributed on the mammalian cell surface. Thus, *H. pomatia* lectin binds to erythrocytes of blood group A but not to erythrocytes of blood groups B and O (9, 14). T-lymphocytes treated with neuraminidase (but not untreated lymphocytes) bind the lectin. B-lymphocytes, on the other hand, do not bind to the lectin, whether or not they are pretreated with neuraminidase (8, 14). The soybean lectin demonstrates affinity for group A and, to some extent, also group B erythrocytes (4, 14). In preliminary studies, we observed that blood group A and B antibodies coupled to protein A-Sepharose both adsorbed the lectin-purified

HSV glycoprotein gC. These findings and their possible biological backgrounds will be studied further.

ACKNOWLEDGMENTS

HSV-specific antisera were kindly supplied by B. F. Vestergaard, University of Copenhagen, Denmark. We thank Inger Sjöblom and Alexandra Sanchez for their skillful technical assistance.

This work was sponsored by the Swedish Medical Research Council (grant 4514), the National Swedish Board for Technical Development (project Kb 5112304-0/2601-3), and the Medical Faculty of Göteborg.

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