Separation of Herpes Simplex Virus-Induced Antigens by Concanavalin A Affinity Chromatography

MANUEL PONCE DE LEON, HELENA HESSLE, AND GARY H. COHEN

Department of Microbiology, School of Dental Medicine, University of Pennsylvania and Center for Oral Health Research, Philadelphia, Pennsylvania 19174

Biologically active herpes simplex virus (HSV)-induced antigens were selectively removed from extracts of infected BHK cells by affinity chromatography by utilizing an insoluble form of concanavalin A (Con A). Soluble extracts of ^{3}H-glucosamine-labeled, HSV-infected cells were absorbed to a Con A column. Bound material was eluted with α-methyl-D-mannoside (αMM) and NaCl. The specific activity of the eluted glycoproteins increased by 10-fold. Two broad groups of viral-induced antigens were isolated from Con A. Group I includes two antigens which bind to Con A by a specific mechanism because the antigens are dissociated by αMM. Group II contains three antigens which bind to Con A but apparently by a nonspecific or electrolytic mechanism. One antigen in group I was identified as the glycoprotein antigen, CP-1, described previously.

Infection of mammalian cells with herpes simplex virus (HSV) elicits the production of a number of virus-induced glycoproteins. Recent estimates indicate that at least 12 new glycoproteins are present in the infected cell (27). Although there has been speculation on the function of these glycoproteins (21, 22), specific information regarding the role of the virus-induced glycoproteins in the infectious process is lacking in most cases. Available evidence indicates that some are viral structural components (3, 18, 19, 27).

Recently, we reported the isolation of an HSV-specific antigen (designated CP-1) that possesses characteristics of a glycoprotein (3). Antibody prepared against this antigen showed a powerful capacity for neutralizing HSV, suggesting that the CP-1 antigen was associated with the viral envelope.

Concanavalin A (Con A), a protein isolated from the jack bean, forms specific complexes with certain polysaccharides and glycoproteins (8, 29, and see review in 24). The reaction between Con A and the carbohydrate-containing molecule is specific for α-D-glycosyl and sterically related residues (9, 20, 25). Recently, affinity chromatographic methods have been described that employ insolubilized Con A columns for the separation and isolation of glycoproteins (1, 7, 10, 13, 32).

To further the study of HSV-glycoprotein antigens, we reasoned that affinity chromatography employing an insoluble form of Con A could be utilized to selectively remove these components from extracts of HSV-infected cells. This report describes a method for separating HSV antigens into two groups: group I antigens that were bound to and eluted from Con A with specific saccharides and group II viral antigens which were eluted with NaCl but not with saccharides.

MATERIALS AND METHODS

Cell cultures. Monolayer cultures of baby hamster kidney cells (BHK) were propagated in 32-oz (0.95 liter) bottles or roller bottles containing Eagle minimal essential medium supplemented with 5% fetal calf serum.

Virus and virus titration. Herpes simplex virus (strain HF) was used. This strain elicits syncytium formation in BHK cells. Preparation of high-titered virus stock as well as assay by plaque titrations were performed as described previously (3).

Soluble antigen preparations. Monolayer cultures of BHK cells were infected at an input multiplicity of 5 to 10 PFU per cell. The cells were harvested after 18 h, and the soluble antigen mixture (SAM) was prepared as described previously (3). Protein concentration was determined by the method of Lowry et al. (14), using crystalline bovine serum albumin as a standard.

Labeling of the cells. Isotopically labeled SAM was obtained from uninfected or infected BHK cells by incubating the culture for 15 h in medium containing either ^{3}H-glucosamine (1.0 μCi/ml; sp act 1 to 2 Ci/mmol) or ^{14}C-valine (0.5 μCi/ml: sp act 35 μCi/mmol). The radioactive label was usually added 3 h after infection of the cells. Newly synthesized HSV-specific proteins are preferentially glycosylated during the 15-h interval (18, 27). Furthermore, 92% of the glucosamine label incorporated into infected cell macromolecules was reportedly recovered in the form of glucosamine and galactosamine (12). All radioisotopes were purchased from New England Nuclear Corp.

Preparation of the insolubilized Con A. A 50-ml (settled volume) column of insolubilized Con A (Glycosylex A trademark, Miles Laboratories) was washed successively with 0.01 M phosphate buffer, pH 7.2 (PB), 0.2 M α-methyl-D-mannoside (αMM) (Sigma Chemical Co.), PB, 2 M NaCl, and PB until no further UV-absorbing material was eluted. The washed material was mixed for 2 h at 36 C with HSV-SAM (usually 1.5 to 3.0 mg of protein per ml), and the column was repacked. Elution was carried out as described in Results.

Immunological techniques. Gel immunodiffusion was performed on Plexiglas templates by the method of Crowle (4), employing 1.0% Ion Agar No. 2 (Oxoid) in 0.05 M Veronal buffer, pH 8.6. After the patterns developed, the plates were washed with 0.2 M αMM in saline to eliminate nonspecific precipitin bands (9, 31). The radial diffusion assay (15) was performed to quantitate the concentration of Con A which was eluted from the insolubilized Con A columns concurrently with glycoproteins. Concanavalin A (3 $\times$ recrystallized) and anti-Con A serum were purchased from Miles Laboratories.

Virus neutralization test. The plaque reduction technique was employed in essentially the same manner as previously described (3). The sera were heated at 56 C for 30 min to inactivate complement. The greatest dilution of serum causing a 50% reduction of the plaque count (on BHK cell monolayers) as compared with virus-preimmune serum mixtures was selected as the neutralizing titer. Details for performing the neutralization test in the presence of Con A will be discussed in Results.

Serum blocking activity. The technique used was essentially that of Zwartouw and Westwood (33). Virus-specific antisera were employed at a dilution capable of reducing the number of HSV PFU by 50%. Extracts prepared from infected or uninfected BHK cells or antigen fractions, at various protein concentrations, were incubated with antiserum for 1 h at 37 C. A known quantity of HSV was then added to the antigen-antibody mixture, incubated for 1 h, and plated on BHK monolayers. The number of PFU formed by this mixture is designated a in the equation which follows. Two controls were also titered: control b which contained the same quantity of HSV and also contained enough anti-HSV serum to neutralize 50% of the virus; and control c which contained the same quantity of HSV and also contained normal rabbit serum. Thus, control b represents the maximum

amount of neutralization (0% blocking) and control c represents the maximum number of PFU possible if there is complete (or 100%) blocking of antibody activity. The capacity of an antigenic preparation to block the virus-neutralizing ability of an antiserum was calculated by the following equation: $[(a - b)/(c - b)] \times 100$ = % of maximum blocking of serum neutralization or blocking ability; when $a = b$, blocking ability = 0%; when $a = c$, blocking ability = 100%.

Amino acid analysis. Samples were hydrolyzed in 6 N HCl at 110 C in vacuo for 18 h. After removal of the acid by evaporation, the basic amino acids, acidic amino acids, and hexosamine fractions were separated and isolated on an amino acid analyzer (Joel, Inc., Medford, Mass.) equipped with an eluent divider.

RESULTS

Affinity chromatography of HSV-SAM on a Con A affinity column. The saccharide-binding properties of an insoluble form of Con A make this carbohydrate exchange resin highly specific for selectively removing glycoproteins from extracts of HSV-SAM. Apparently only molecules containing terminal glycosyl residues are bound specifically by this resin. Elution of the bound material, presumably glycoproteins, can be achieved by displacement with either of the simple saccharides, αMM or α-methyl-D-glucoside (αMG), which have higher binding affinities for the Con A than the bound carbohydrate residues (9, 20, 25).

Figure 1 illustrates a typical elution profile of HSV-SAM protein (^{14}C-valine) and glycoprotein (^{3}H-glucosamine) removed from the affinity column with different developing solutions. The distribution of carbon label (protein) was closely correlated with the elution pattern of tritium label (carbohydrate). Separate experiments were performed using only ^{3}H-glucosamine-labeled SAM (sp act 4 $\times$ 10^4 counts per min of ^{3}H-glucosamine per mg of protein). The eluates from these experiments were pooled into four fractions as indicated in Fig. 1, dialyzed against PB, and concentrated. The first peak fraction, designated Con A-SAM (sp act 10^4 counts per min per mg of protein) corresponds to the unbound protein washed from the column with PB. The second fraction, designated group I (sp act 3.6 $\times$ 10^5 counts per min per mg of protein) contained the peak of radioactivity eluted from the column with 0.2 M αMM. The column was washed free of αMM with PB, and the third fraction, designated group II (sp act 9 $\times$ 10^4 counts per min per mg of protein) was eluted with 2 M NaCl.

These results suggest that Con A affinity chromatography selectively binds glycoproteins from HSV-SAM and, after elution with saccha-
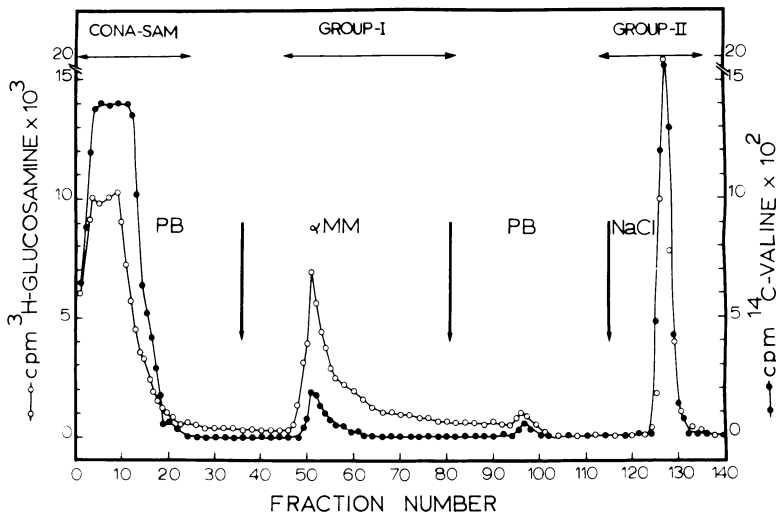
FIG. 1. *Elution profile for the Con A affinity chromatography of HSV-SAM. The Con A column (50 ml) was prepared and developed at room temperature as described in Materials and Methods. The flow rate of the column was 60 ml/h. Fractions of 5 ml were collected. The specific activity of ³H-glucosamine-SAM was 3 × 10⁴ counts per min per mg (O) and ¹⁴C-valine-SAM 3 × 10³ counts per min per mg (●), respectively. The arrows indicate the change of the elution solutions. Abbreviations: PB, 0.01 M phosphate buffer, pH 7.2; αMM, 0.2 M α-methyl-D-mannoside; and NaCl, 2 M NaCl.*

rides, the specific activity of these moieties is increased in the concentrated pooled fractions approximately 10-fold. However, the group II fraction contained a relatively lower level of glucosamine label, indicating that it may contain components other than glycoproteins. Since this fraction was not eluted from Con A by saccharides, the components in it appear to bind to the column by a nonspecific mechanism (2, 5, 6).

It is possible that the nonspecific attachment of the group II fraction (or the other fractions) to Con A was by some mechanism other than Con A carbohydrate binding. Conceivably, not all of the label found in each Con A glycoprotein fraction was contained in carbohydrate residues. The tritium-labeled glucosamine may have been metabolically converted to amino acids and then to protein during the 15-h incubation period. To check this possibility, samples of ³H-glucosamine-labeled group I and group II fractions were hydrolyzed and the basic amino acids, acidic amino acids, and hexosamine fractions were separated and isolated on an amino acid analyzer. More than 88% of the label in each fraction was in the hexosamine fraction. Keller et al. (12) reported that 92% of the glucosamine label incorporated into infected cell macromolecules was recovered in the form of glucosamine and galactosamine. We conclude that the radioactive labeling pattern

for both fractions truly reflects the attachment and elution of carbohydrate residues from Con A.

Pertinent information concerning Con A affinity chromatography of HSV-SAM. (i) More than 90% of the input radioactive counts were routinely recovered in these experiments. No further radioactivity was removed by treatment of the column with αMG nor with 0.1 N HCl which destroys the binding capacity of Con A. This indicates that all of the radioactive glycoproteins were removed by αMM and NaCl treatment. (ii) The Con A column still retained its binding capacity and suffered no apparent loss of specificity after being used several times. (iii) Even though the column was exhaustively prewashed, small amounts of Con A were found in each of the eluted fractions. Con A was detected by gel diffusion employing anti-Con A serum. (iv) When the Con A-SAM fraction was collected, concentrated, and recycled through the insolubilized Con A column, approximately 20% of the counts were bound to the column. We conclude that the column did not have the capacity to absorb all of the glycoprotein from SAM in a single run. (v) Uninfected BHK cells were labeled for 15 h with ³H-glucosamine, and the extract was chromatographed on Con A. The elution profile for glycoprotein was virtually identical to that shown in Fig. 1. The specific activities (counts per minute of ³H-

glucosamine per milligram of protein) of the fractions were as follows: uninfected group I, 2.2 $\times$ 10^5; group II, 8.5 $\times$ 10^4. Apparently, BHK contains receptors which bind to Con A and elute in a manner similar to infected cell extracts. Therefore, it is likely that the Con A fractions obtained from infected cells contain both viral and host cell glycoproteins.

Characterization by gel diffusion of the Con A affinity column fractions. A complex pattern of six viral-induced immunoprecipitin bands was previously obtained when SAM, prepared from BHK cells, was tested with anti-SAM serum (3). When Con A-SAM was tested with anti-SAM serum, the same complex immunoprecipitin pattern was obtained. Figure 2A shows the reaction of the pooled group I and group II (by gel diffusion) with anti-SAM serum. The group I fraction, eluted by mannoside, contained two viral-induced precipitin bands; one of these was a heavy band (Fig. 2B, symbol *b*) and the other (symbol *a*) was somewhat weaker. The group II fraction, eluted with NaCl, contained three bands; two of these, *c* and *e*, were strong, and the third, *d*, was faint. The pattern in Fig. 2A suggests that none of the three bands in group II showed identity with either band in group I. This conclusion was strengthened by additional gel diffusion tests in which the concentrations of reactants were varied. No immunoprecipitin bands were observed when uninfected BHK cell fractions were reacted against absorbed anti-SAM serum. These results are interpreted as follows: the viral-induced antigens can be divided into two broad groups on the basis of the type of binding to Con A. Group I includes two antigens which have a high affinity for Con A. They bind to the lectin by a highly specific mechanism which is dissociated by saccharides (9, 20, 24, 25). On the other hand, group II contains antigens which have an affinity for Con A, but which bind by some other mechanism (24). This is evidenced by the observation that the antigens in group II remained bound to Con A even in the presence of 1.0 M αMM or αMG, but were eluted from the lectin by 0.3 M NaCl.

Relationship between CP-1 antigen and the antigens eluted from the Con A affinity column. We recently reported the isolation of a viral-specific antigen, designated CP-1, from a mixture of viral-induced antigens by chromatography on calcium phosphate (3). This antigen, which appears to be a glycoprotein, also appears to be a structural component of the virus envelope. Experiments were designed to determine the relationship of CP-1 to the antigens isolated from the Con A affinity column.
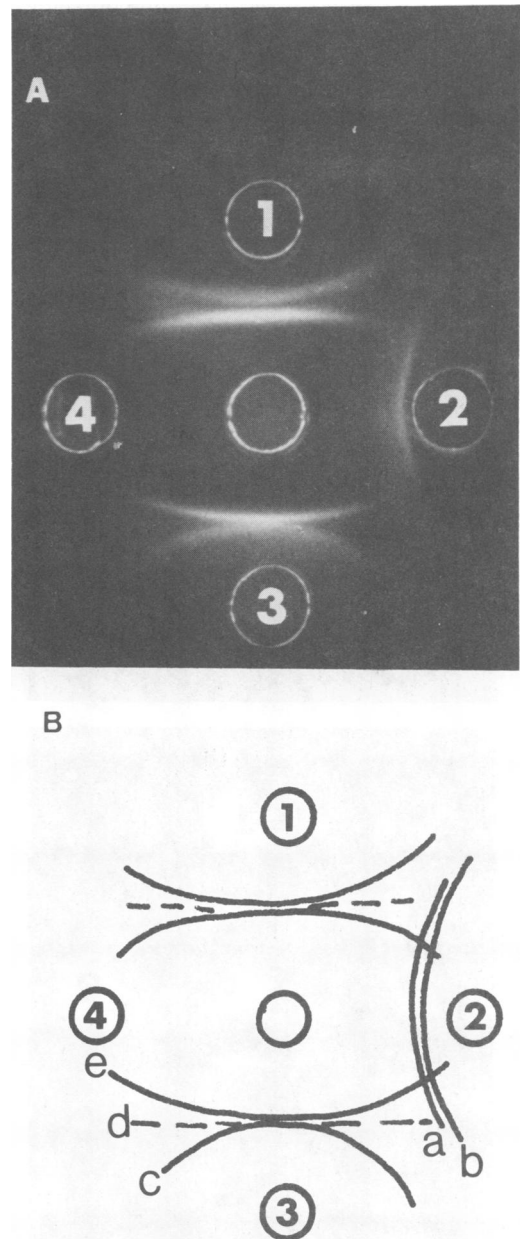

FIG. 2. A, Gel diffusion analysis of the individually pooled column fractions obtained by Con A affinity chromatography of HSV-SAM. Center well, Anti-HSV-SAM serum. Peripheral wells: 1, group II (protein concentration 1.8 mg/ml); 2, group I (protein concentration 1.2 mg/ml); 3, group II (same as 1); 4, uninfected BHK extract (8.2 mg/ml). B, Diagrammatic representation of A.

We found that, by selectively pooling the peak fractions of group I (Fig. 1, tubes 48–52), we could isolate antigen *b* so that it was

apparently free of the second immunoprecipitin (a). This separation of antigen a and b is shown in Fig. 3A employing anti-SAM serum to detect the antigens in the peak fraction and in the remaining tubes (53–82) of group I.
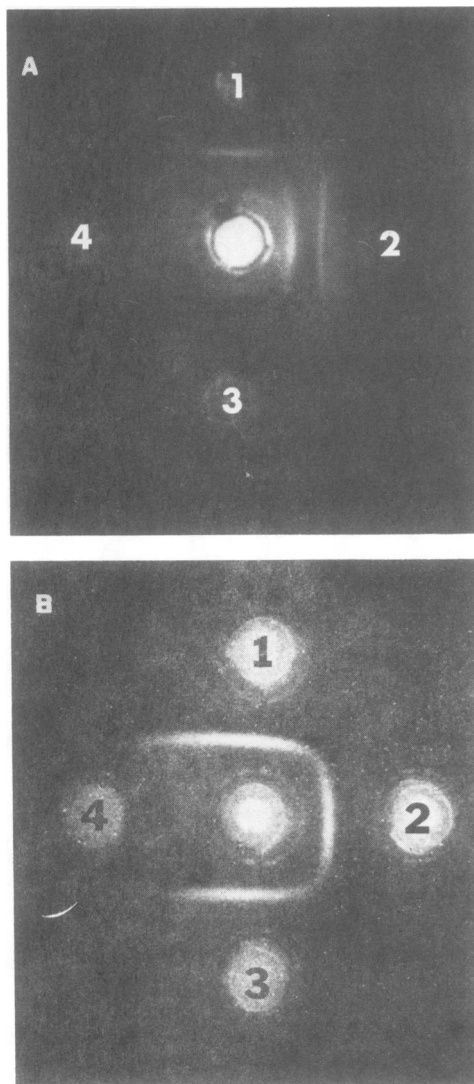

FIG. 3. A, Gel diffusion analysis of the separation of the group I antigens. Center well, Anti-SAM serum. Peripheral well: 1, peak fractions of group I (pooled fractions 48 through 52); 2, trailing edge of group I (pooled fractions 53 through 82). B, Gel diffusion analysis of fractions eluted from the Con A column and their relation to CP-1 antigen. Center well: anti-CP-1 serum. Peripheral wells: 1, HSV-SAM (protein concentration 5 mg/ml); 2, CP-1 antigen (0.3 mg/ml); 3, antigen b (0.3 mg/ml) and group I antigen from uninfected BHK cells (0.4 mg/ml).

We then tested the relationship between antigen b and antigen CP-1 (the latter prepared from the immunosorbent column as in reference 3). Figure 3B shows the gel diffusion pattern obtained when anti-CP-1 serum was reacted against HSV-SAM, CP-1 antigen, antigen b from infected cells, and the group I fraction obtained from uninfected BHK cells. One band of identity was observed in the infected cell preparations. This indicates that CP-1 and group I (antigen b) contained the same immunoprecipitin and provides further evidence that the CP-1 antigen is a glycoprotein. We are presently trying to separate antigen a from group I and determine its function.

Blocking of the neutralizing capacity of antiserum by Con A preparations. We previously showed that the CP-1 antigen stimulates the production of virus neutralizing antibody (3). If antigen b isolated from Con A possesses the same biological activity as the CP-1 antigen of an immunosorbent (3), then it should be capable of combining with anti-CP-1 or anti-SAM serum and thus could block the virus neutralizing capacity of these sera. However, it was first necessary to determine the effect of Con A (present as a contaminant in the antigen preparation) on the serum-blocking assay. Two reported properties of Con A might complicate the results. First, it has been shown that HSV has receptor sites for Con A and, upon exposure to the lectin, the virus became noninfectious (17); this phenomenon was reversed by incubating the virus in saccharides (αMM). Secondly, it has been shown that Con A forms precipitates with serum proteins (16, 32). The first reaction would tend to lower the PFU number in our assay, whereas the second would tend to raise the PFU number. To test this, we added different concentrations of Con A to 100 PFU of HSV in the presence and absence of 0.04 M αMM. The mixture was incubated at 37 C for 1 h, and the number of remaining PFU was determined. Table 1 shows that 1 μg of Con A per 100 PFU reduced the number of plaques by 16%. The presence of higher concentrations of Con A led to greater reductions in infectivity. As demonstrated previously (17), we also found the artifacts created by Con A were eliminated by adding 0.04 M αMM to the assay mixture. Thereafter, αMM was included in all serum blocking assays of Con A column fractions.

Table 2 shows that antigen b (36 μg of protein per ml) completely blocked the neutralizing activity. In contrast, antigens in the group II fraction (112 μg of protein per ml) failed to block the neutralizing activity. In comparison

with these results, it is interesting to note that as little as 10 μg of CP-1 was adequate to completely block neutralizing activity, whereas 840 μg of protein from uninfected BHK cells had no effect. These results suggest that antigens b and CP-1 share the same biological activity as well as the same properties in gel diffusion. These results also suggest that the antigens in group II and CP-1 do not share the same biological properties.

Analysis of the viral-induced antigens found in the Con A NaCl fraction. Figure 4A shows the gel diffusion pattern when anti-SAM and anti-CP-1 serum were reacted against the concentrated group II fraction. Three immunoprecipitin bands were observed with anti-SAM serum and none with anti-CP-1 serum.

TABLE 1. *Effect of various concentration of Con A on herpes simplex virus infectivity in the presence and absence of α methyl-D-mannoside (αMM)*

Concn of Con A (μg/ml)	% Inhibition of infectivity by Con A[a]	
	αMM absent	αMM[b] present
0	0	0
1	16	0
5	35	0
10	52	0
20	82	0
50	100	0

[a] 0%, No inhibition of infectivity; 100%, complete inhibition of infectivity, i.e., no PFU observed.

[b] Concentration of 0.04 M was employed; higher concentrations of αMM, i.e., 0.2 M, was toxic to the cells.

Calcium phosphate chromatography was employed previously to separate CP-1 antigen from the remaining viral antigens (3). In these studies, a major viral-specific immunoprecipitin band was eluted by PB at concentrations greater than 0.06 M. This antigen was designated CP-4. Figure 4B shows the gel diffusion pattern obtained when anti-CP-4 serum was reacted against group II, SAM, uninfected BHK-group II, and a SAM extract from the Savage strain of HSV (type 2) (virus obtained from L. Howard Moss). These results suggest that CP-4 and group II contain a common virus-specific antigen. (Other experiments showed that this antigen corresponds to antigen e in Fig. 2B). Furthermore, this antigen appears to be common to both type 1 and type 2 HSV.

Binding of CP-1 antigen to Con A in the presence of Nonidet P-40. This study has provided additional evidence that the CP-1 antigen is a glycoprotein. However, one must recognize the possibility that CP-1 is closely associated with cellular membranes and glycoproteins. Thus, it may be argued that CP-1 is not a glycoprotein but binds to Con A because of its association with cellular products. For example, sonic treatment of infected cells creates small pieces of membrane (micro membrane vesicles) not sedimented by high-speed centrifugation (28). Host cell products could bind specifically to Con A and fortuitously carry along the CP-1 antigen.

The following experiments were performed to show that CP-1 antigen binds specifically to Con A and that this binding is apparently not dependent upon the presence of associated

TABLE 2. *Blocking of serum-neutralizing capacity by different antigen preparations[a]*

Antigen	Protein (μg/ml)	Con A[b] concn (μg)	Anti-SAM serum (% blocking)[c]	Anti-CP-1[d] serum (% blocking)
HSV-soluble antigen mixture	100		100	100
CP-1 antigen[d]	10		100	100
Uninfected BHK cell extract	840		0	0
Antigen b (isolated from infected BHK)	36	0.50	100	100
	18	0.25	47	44
	9	0.125	21	18
Group II	112	1.0	0	0

[a] All experiments were carried out in the presence of 0.04 M αMM.

[b] Parentheses indicate the μg of Con A present in the various fractions as determined by radial diffusion assay (15).

[c] 0%, No blocking of serum-neutralizing capacity; 100%, complete blocking of serum-blocking capacity.

[d] Anti-CP-1 serum and CP-1 antigen were prepared as previously described (3).
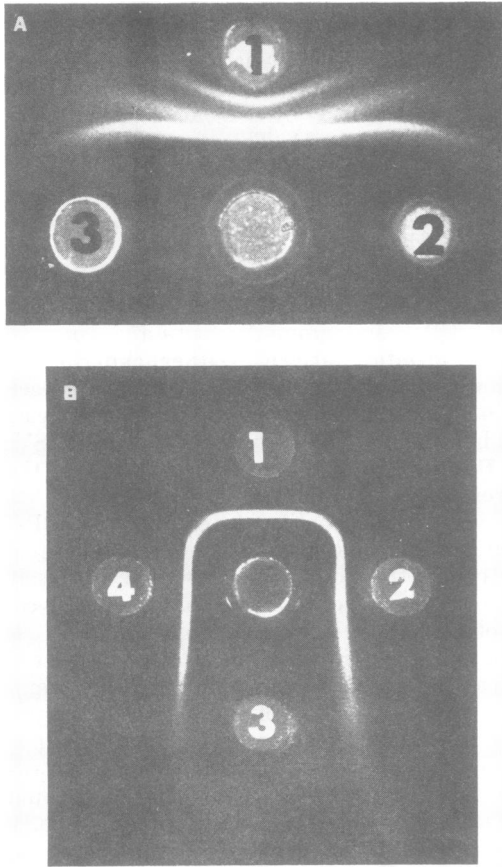
FIG. 4. *Gel diffusion analysis of fractions eluted from the Con A column. A, Center well, group II (protein concentration 0.9 mg/ml). Peripheral wells: 1, anti-HSV-SAM serum; 2 and 3, anti-CP-1 serum. B, Center well, anti-CP4 serum. Peripheral wells: 1, group II (protein concentration 0.9 mg/ml); 2, HSV-SAM (5 mg/ml); 3, group II antigen from uninfected BHK cells (0.8 mg/ml); 4, HSV-SAM from Savage strain of HSV type 2 (4 mg/ml).*

membrane. We tested the effect of the nonionic detergent Nonidet P-40 (NP-40) on the antigenic activity of CP-1 and on the Con A elution pattern of HSV-SAM. This detergent was used because of its lipid-protein dissociation properties and on the basis of reports that lipid-free protein obtained with NP-40 retained the immunological properties of the native proteins (11, 23). Gel diffusion analysis showed that neither the antigenic activity of CP-1 nor that of the other viral antigens was destroyed by NP-40 treatment.

When ^{3}H-glucosamine-labeled HSV-SAM and ^{14}C-valine-labeled HSV-SAM were solubilized with 0.5% NP-40 (37 C for 1 h) and

co-chromatographed on the NP-40-modified Con A column, the radioactive elution profile was virtually identical to that observed in Fig. 1. For these experiments the Con A column and all of the developing solutions were prepared in 0.1% NP-40 to prevent aggregation of proteins or reassociation of lipid and protein. The group I and II fractions were collected as indicated in Fig. 1, dialyzed, and concentrated. The gel diffusion pattern of the antigens isolated from the NP-40-modified column was unaltered.

We conclude from these experiments that the CP-1 antigen binds specifically to Con A by virtue of carbohydrate residues on the viral antigen. Furthermore, it is also unlikely that group II antigens represent membrane-bound proteins which were physically trapped in the insoluble matrix and then released by elution with NaCl (23). It appears that the attraction of group II antigens to Con A is the result of a direct electrostatic effect.

DISCUSSION

Our study provides a method for separating HSV-induced antigens synthesized after infection of BHK cells into two groups. This separation involves the use of a lectin with highly specific saccharide-binding properties (9, 20, 24, 25) and less specific binding properties as well (2, 5, 6). Infected cell extracts were mixed with insolubilized Con A, and the two groups of antigens were eluted. Group i, designated the specific binding fraction, contained two antigens that were bound to and eluted from Con A with αMM. The specific binding properties of Con A have been extensively studied by Goldstein et al. (9, 20, 25). The binding of sugar residues to Con A requires the C-3, C-4, and C-6 hydroxyl groups of the α-D-mannopyranoside, α-D-glycopyranoside, or N-acetyl glucosamine. This suggests that at least some of the terminal carbohydrate residues of the viral antigens in group I may be one or more of these moieties. Our observation that group I antigens can be dissociated from Con A at low saccharide concentrations (0.04 M αMM) suggests that the terminal end of the oligosaccharide in the antigens does not have repeating residues of α-D-glucose or α-D-mannose (26).

Our results show that the Con A binding properties of the specific antigens were unaffected by prior treatment of HSV-SAM with 0.5% NP-40 and chromatography of the dissociated material in the presence of NP-40. We interpret this to mean that host receptors on cellular membranes which also bind specifically to Con A are not involved in the binding of viral

antigens to Con A. We conclude that the group I antigens bind directly to the Con A by virtue of a carbohydrate residue on the viral antigens.

By carefully selecting fractions from the group I peak, we separated one of the two viral immunoprecipitins. This immunoprecipitin (antigen b) was identified as the glycoprotein CP-1 antigen described previously (3). Both the CP-1 antigen isolated from an immunosorbent and antigen b share the same biological activity for the blocking of serum neutralizing activity and are identical in gel diffusion. Furthermore, the CP-1 antigen and the antigen b are both eluted from calcium phosphate by 0.04 M phosphate (unpublished results). Parenthetically, the CP-1 antigen has been shown recently to be identical to the band II antigen described by Watson and Wildy (3) (C. Sim, D. H. Watson, M. Ponce de Leon, and G. Cohen, unpublished data). In addition the CP-1 antigen appears to be present in both HSV type 1 and type 2 (G. Cohen and M. Ponce de Leon, unpublished data).

In a previous report, we presented evidence that the viral structural component CP-1 is, in part, glycoprotein (3). Our current findings that the CP-1 antigen binds specifically to Con A is further evidence that this viral structural surface component is a glycoprotein. We also suggested previously that the CP-1 antigen may be responsible for some or all of the altered biological properties associated with the HSV-infected cell membrane (3). In this connection, Tevethia et al. (30) detected early cell surface changes in HSV-infected cells employing the property of Con A to bind to and agglutinate infected but not uninfected cells. It is not clear whether or not the viral glycoproteins are related to the "new" Con A agglutinating moiety found on the cell surface. However, the new Con A binding moiety does share two features in common with the group I antigens: one is the propensity of group I antigens to bind to Con A and the second is the location of one of the group I antigens, CP-1-antigen b, on the envelope of the virus. Infected cells acquire new determinant antigens on their surfaces which are similar to or identical with those on the surface of the herpes virion (22). However, further work is required before any conclusions can be drawn.

The second major group of glycoprotein antigens which bind to Con A has been designated group II or the nonspecific fraction since they are eluted with NaCl but not with saccharides. The group II fraction contained three viral immunoprecipitin bands not found in group I.

This conclusion was based on gel diffusion analysis. In support of this conclusion, we also found that the NaCl fraction had no effect on serum-blocking activity. These data suggest that, if the group II antigens represent viral structural components, they should be located in a subsurface position in the virion relative to the CP-1 antigen.

By what mechanism do the group II antigens bind to Con A? Group I and group II antigens are eluted from Con A by different compounds, suggesting a difference in the nature of the binding of these antigens to Con A. This difference is most likely to be reflected in the carbohydrate composition of the two fractions. Con A binds to neutral polysaccharides and these complexes are stable over a wide range of salt concentrations which negate any electrostatic interaction (25). However, Con A also binds to and may precipitate polyelectrolytes and polysaccharides which are devoid of terminal mannopyranoside (or related) residues (2, 5, 6).

Several alternative explanations are possible for the binding of group II glycoprotein antigens to Con A. For example, carbohydrates in group II antigens may possess terminal sequences of several repeating α-$(1\rightarrow 2)$-D-mannopyranosyl residues. It has been shown that these repeating molecules possess a greater binding capacity for Con A, and therefore greater concentrations of saccharides are required to dissociate them from Con A (26). However, this does not appear to be the case since we could not dissociate the group II antigens from Con A with 1 M αMM, whereas they did dissociate readily in the presence of 0.3 M NaCl. Secondly, our studies with NP-40 indicate that it is unlikely that the group II antigens represent membrane-bound moieties which become physically trapped in the inert matrix. The most reasonable hypothesis is that the three antigens are associated covalently or electrostatically with a polyelectrolyte which binds the antigens to Con A by a nonspecific or electrolytic mechanism. Experiments are now in progress to explore these relationships.

We conclude that there are at least five major viral immunoprecipitins which are associated with carbohydrate in extracts of HSV-infected BHK cells. These antigens are immunologically active and at least one, the CP-1 antigen, retains its biological activity after separation by Con A affinity chromatography.

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