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Glycoprotein gp118 of Varicella-Zoster Virus: Purification by Serial Affinity Chromatography

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Glycoprotein gp118, one of the major glycosylated proteins specified by varicella-zoster virus, is biologically of great importance since it possesses an epitope which elicits a complement-independent neutralizing antibody response. To purify this glycoprotein from a Nonidet-solubilized extract of varicella-zoster virus-infected cells, we examined its affinity to a variety of ligands, including two lectins—concanavalin A and *Lens culinaris*, Cibacron blue and heparin, and finally an immunoadsorbent anti-gp118 monoclonal antibody. By serial affinity chromatography on three different columns consisting of, respectively, (i) Cibacron blue dye-Sepharose, (ii) *L. culinaris*-Sepharose, and (iii) anti-gp118 murine monoclonal antibody bound to CNBr-activated Sepharose, we isolated varicella-zoster virus-specific gp118 essentially free of contamination by any other radiolabeled viral or cellular polypeptide. The fold purification was estimated at 1,025 and the percent recovery at 13.6. On the basis of its chromatographic properties, gp118 appeared to contain mainly asparagine-linked, biantennary, complex-type, and hybrid-type oligosaccharides.

Varicella-zoster virus (VZV) is one of the more reclusive members of the human herpes group viruses. Known mainly as the etiological agent of the common childhood exanthem chickenpox, the virus usually remains in a latent state for several decades thereafter until late adulthood when activation manifesting itself as the dermatomal disease zoster becomes an increasingly common event (13). Unlike the prototype herpes simplex virus and to a lesser extent cytomegalovirus, in vitro infection with VZV is nearly completely cell associated; very few complete infectious virions are released into the medium overlying an infected monolayer (12, 27). In cultured human cells the VZV genome codes for at least three major glycoproteins which have been designated gp62, gp98, and gp118 according to their estimated molecular weights ($\times 10^{-3}$) as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis under reducing conditions (8, 10). These three glycosylated polypeptides also are present in the envelope of the gradient-purified virion (11, 24, 25).

Since further characterization of the individual viral glycoproteins was hindered by lack of monospecific antisera, we employed somatic cell fusion technology to produce murine hybrid cell lines which secreted antibody directed against the VZV-induced glycosylated antigens. Our initial panel included five monoclones: one precipitated only gp118, and the other four reacted with both gp98 and gp62 (9). The anti-gp118 antibody reduced VZV plaque formation by greater than 80%, whereas the four hybridoma secretory products directed against gp62 and gp98 failed to exhibit similar neutralizing activity. In this communication, we describe the fractionation and purification of the biologically important glycoprotein gp118 on a series of affinity columns which include the immunoadsorbent monoclonal antibody together with other immobilized ligands.

Monoclonal antibody clone 9C6. The derivation of murine monoclonal antibody (clone 9C6, isotype immunoglobulin G1) directed against VZV-specific glycoprotein gp118 has been described in a recent publication (9). In Fig. 1, the electrophoretic profiles of three VZV-infected cultures isotopically labeled with [^{3}H]mannose, [^{14}C]glucosamine, and [^{3}H]fucose are illustrated, as well as those of the respective precipitates, after reaction of the three antigen preparations with the monoclonal antibody. In each instance, a single band was observed in the immunoprecipitate. The [^{3}H]mannose- and [^{14}C]glucosamine-labeled glycoproteins corresponded in electrophoretic mobility with that of the previously identified [^{3}H]fucose-labeled glycoprotein gp118 (9). The larger number of glycosylated polypeptides labeled with [^{14}C]glucosamine in the unprecipitated antigen preparation included glycoproteins encoded by both cellular and viral genomes, whereas [^{3}H]fucose was incorporated almost exclusively into virus-specific glycoproteins under our conditions of infection and isotopic labeling (8).

Chromatography on immobilized lectins. A prior publication has suggested that purification of a single viral glycoprotein from an infected cell extract by a specific immunoadsorbent may require several repetitive applications before radiochemical homogeneity is achieved (5). We wished to determine whether preliminary chromatography on other immobilized ligands could remove the majority of the undesired proteins and diminish the need for more than one immunoaffinity chromatogram. Because other herpes group viral glycoproteins have been purified by concanavalin A (ConA)-Sepharose chromatography (2, 21, 22, 28), we initially evaluated this lectin and a second plant product *Lens culinaris*, which exhibits similar but lower affinities primarily for mannose and glucose residues (6, 18). The lectins were coupled to Sepharose 4B (Pharmacia Fine Chemicals) previously activated with cyanogen bromide as originally described by Cuatrecasas (3). The quantities of ConA and lentil protein bound in the columns were 6.3 and 7.4 mg/ml of settled gel, respectively. Samples of [^{14}C]glucosamine-labeled VZV-infected cell extracts were chromatographed on both ConA- and lentil-Sepharose 4B columns. The elution profiles illustrated in Fig. 2 demonstrated a large amount of radioactivity in the void volume followed by a second peak after desorption with 0.5 M α-methylmannoside (α-MM). In agreement with results of other investigators, we increased our recoveries of viral glycoproteins from lentil columns by the addition of a sulfobetaine emulsifying agent (SB14)

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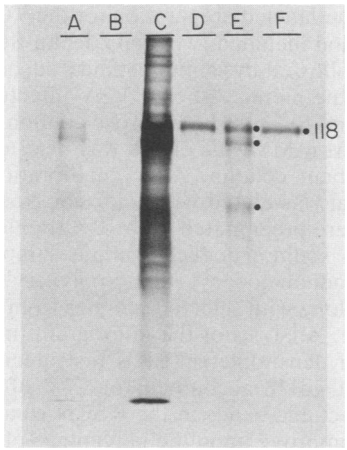

FIG. 1. Antigenic specificity of monoclonal antibody clone 9C6. Samples (25 μl) of ascites monoclonal antibody 9C6 were reacted with Nonidet-solubilized extracts (200,000 to 300,000 cpm) of VZV-infected cells which had been intrinsically radiolabeled with either D-[2-^{3}H]mannose, D-[*U*-^{14}C]glucosamine, or L-[5,6-^{3}H]fucose, and radioimmune precipitation was performed as previously described (8–10). The immunoprecipitates were analyzed on a 10% polyacrylamide slab gel cross-linked with *N,N'*-diallyltartardiamide at a 37.5:1 ratio by standard sodium dodecyl sulfate-polyacrylamide gel electrophoresis techniques (8, 19). The slab gels were subjected to fluorography (1) and exposed to Kodak XAR-5 or XRP-5 film at −70°C from 4 to 21 days. Lanes A, C, and E contain antigen stocks radiolabeled with [^{3}H]mannose (25,000 cpm), [^{14}C]glucosamine (12,000 cpm), and [^{3}H]fucose (25,000 cpm), respectively, and the corresponding radioimmune precipitates were added to adjacent lanes B (11,000 cpm), D (800 cpm), and F (24,900 cpm). The [^{3}H]fucose-labeled gp118 is designated in the margin to the right of lane F; the two lower-molecular-weight VZV-specific glycoproteins gp98 and gp62 are designated by closed circles in lane E. A faint [^{3}H]mannose-labeled protein corresponding in molecular weight to gp118 is detectable in lane B.

(Zwittergent; Calbiochem-Behring) to the elution buffer (7, 23).

Recoveries of radiolabeled proteins were calculated for each affinity column. For ConA-Sepharose 4B, 53% of the input counts per minute bound to the gel; 34% of this amount was competitively eluted. In the case of *L. culinaris*-Sepharose 4B, 57% of the initial sample adsorbed the column, and nearly half (48%) was desorbed by 0.5 M α-MM. Alterations in the ionic strength of the eluting buffer (1 M NaCl) or the addition of a polarity reducing agent (50% ethylene glycol) did not effect further desorption from either column. Decreasing the concentration of immobilized lectin by substitution with underivatized Sepharose also did not substantially alter the elution profiles of either affinity adsorbent. Electrophoretic analysis of the eluates from the two lectin adsorbents disclosed differences in the glycoprotein profiles (Fig. 2). All three major VZV-specific glycosylated polypeptides—gp62, gp98, and gp118—bound to both plant lectins; however, gp62 was not eluted from immobilized ConA by any of the eluants tested. In contrast, desorption of all three major glycoproteins was easily effected from *L. culinaris*. Chromatography on the latter lectin also enhanced the recovery and confirmed the existence of less conspicuous VZV-specific glycosylated species, e.g., gp88 and gp45, from the detergent-solubilized extract.

Chromatography on heparin-Sepharose and Affi-Gel blue. In addition to lectin chromatography, we also fractionated the VZV-specific glycoproteins on the basis of their electrostatic and hydrophobic interactions with two ligands, blue dye and heparin (15, 17, 26). Samples containing 2×10^5 cpm of [^{14}C]glucosamine were loaded onto the heparin-Sepharose column; the amount of radioactivity which bound to the gel was 33% of the input (Fig. 3). An increase in the ionic strength of the starting buffer solution to 1 M NaCl effected a 39% elution of adsorbed proteins. Further attempts to remove bound counts per minute by the addition of 50% ethylene glycol or dipolar ionic detergents were unsuc-

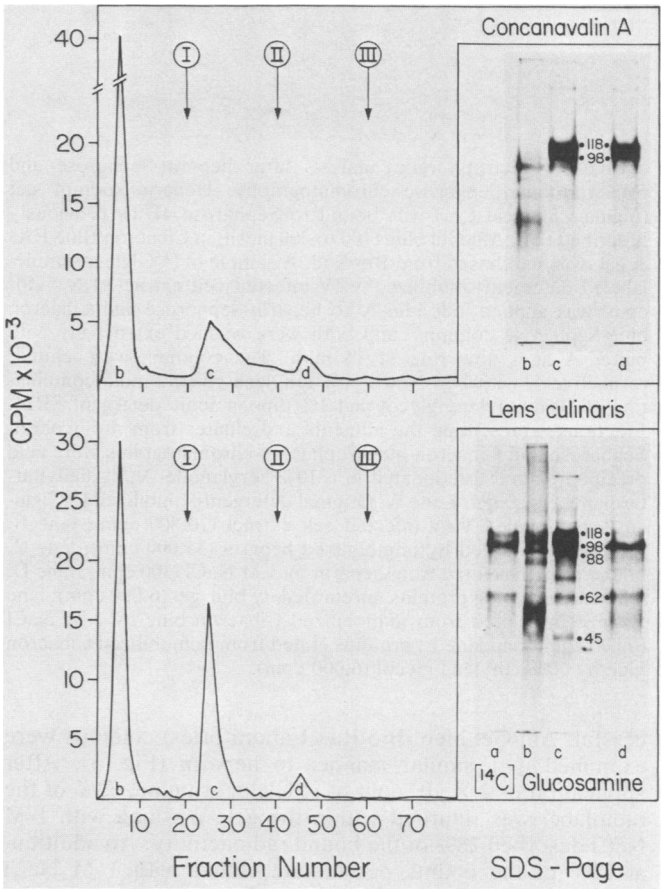

FIG. 2. Fractionation of detergent-solubilized VZV-infected cell extracts by affinity chromatography on immobilized ConA and *L. culinaris* (lentil). A Nonidet-solubilized sample containing 200,000 cpm of [^{14}C]glucosamine-labeled VZV antigen was applied to a lectin-Sepharose column at ambient temperature and allowed to adsorb for 20 min. The column was washed with equilibration buffer A (0.05 Tris (pH 7.6), 0.15 M NaCl, 0.5% Nonidet 40, 0.02% Na azide) at a flow rate of 15 ml/h until the counts per minute returned to baseline. At that time (I), an elution buffer (buffer A + 0.5 M α-MM) was substituted. At time point II, a second elution buffer (buffer A containing 0.5 M α-MM and 1% SB14 detergent) was added. On the following day (time point III), elution was continued with the second buffer until the recovery of the counts per minute fell to baseline levels. The elution profiles for ConA-Sepharose and lentil-Sepharose are represented on the left side of the figure. Fractions b, c, and d containing the peaks of radioactivity were pooled; the proteins were acid precipitated and subjected to electrophoresis in 10% acrylamide–*N,N'*-diallyltartardiamide slab gels (right side). Lane a, Initial VZV extract before chromatography (10,800 cpm); lane b, proteins not retarded by the immobilized lectins (6,000 cpm per lane); lane c, bound proteins which were eluted by α-MM (6,000 cpm per lane); and lane d, proteins desorbed after an overnight incubation in the presence of α-MM and SB14 (4,500 cpm per lane).

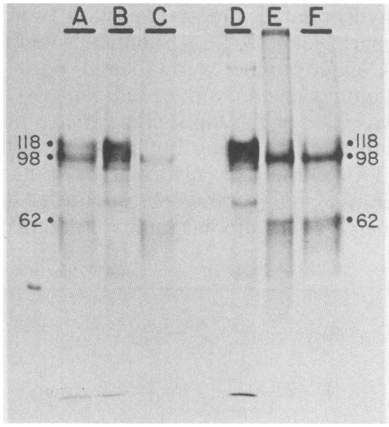

FIG. 3. Electrophoretic analysis after heparin-Sepharose and Cibacron blue-Sepharose chromatography. Heparin sodium salt (Sigma Chemical Co.) was bound to Sepharose 4B as previously described (20). Affi-Gel blue (100 to 200 mesh), a Cibacron Blue F3G A gel, was purchased from Bio-Rad. A sample of [^{14}C]glucosamine-labeled detergent-solubilized VZV-infected cell extract (1.6×10^5 cpm) was applied individually to heparin-Sepharose and Cibacron blue-Sepharose columns, and both were washed extensively with buffer A at a flow rate of 15 ml/h. The columns were eluted, respectively, with 1 M NaCl, 50% ethylene glycol, and a combination of 50% ethylene glycol and 1% dipolar ionic detergent SB14. Fractions representing the effluents and eluates from the heparin-Sepharose and Cibacron blue-Sepharose chromatograms were acid precipitated and fractionated in a 10% acrylamide–*N*,*N*′-diallyltartardiamide slab gel. Lane A, Original detergent-solubilized [^{14}C]glucosamine-labeled VZV-infected cell extract (10,800 cpm); lane B, proteins unretarded by immobilized heparin (35,000 cpm); lane C, bound proteins eluted from heparin by 1 M NaCl (300 cpm); lane D, effluent containing proteins unretarded by blue gel (6,000 cpm); lane E, proteins eluted from immobilized Cibacron blue by 1 M NaCl (6,000 cpm); and lane F, proteins eluted from immobilized Cibacron blue by 50% ethylene glycol (6,000 cpm).

cessful. Affi-Gel blue (Bio-Rad Laboratories) columns were examined in a similar manner to heparin (Fig. 3). After application of 2×10^5 cpm of [^{14}C]glucosamine, 73% of the radiolabel was retarded within the gel. Washing with 1 M NaCl desorbed 28% of the bound radioactivity. An additional 26% of the counts per minute eluted with 1 M NaCl containing 50% ethylene glycol, an effect not observed in the previous experiment with heparin-Sepharose gel. As illustrated in Fig. 3, the electrophoretic profiles representing the radiolabeled glycoproteins in the void volumes and eluates from the two columns were very similar. In neither of the two gels was glycoprotein gp118 retarded, whereas both of the lower-molecular-weight glycoproteins, gp62 and gp98, were relatively enhanced in the 1 M NaCl eluate. Thus, chromatography on either of these two columns effectively isolated gp118 from the two other major VZV glycoproteins.

Immunoaffinity chromatography. Two different serial affinity chromatographic schema, both of which included immunoaffinity adsorption as the final step, were evaluated for improved isolation and purification of glycoprotein gp118. The immunoadsorbent was prepared from ascites monoclonal antibody anti-gp118 which was concentrated by precipitation with saturated $(NH_4)_2SO_4$ and extensively dialyzed against 0.001 M phosphate buffer (pH 7.5). After adjustment of the protein concentration to 10 mg/ml, the monoclonal antibody was coupled to CNBr-activated Sepharose by the method of Cuatrecasas (3). To better assess the purity of the final product, we substituted a [^{35}S]methionine-labeled preparation for the ^{14}C-amino sugar. The first method included two steps: lectin-Sepharose chromatography followed by immunoaffinity adsorbents. A sample of a [^{35}S]methionine-labeled VZV-infected cell extract was applied first to a lentil-Sepharose column. After desorption with 0.5 M α-MM, the eluate was next applied onto an immunoadsorbent column. After equilibration for 60 min, bound material was eluted with 3 M KSCN. Proteins in the final eluate were precipitated with 10% trichloroacetic acid, solubilized in sodium dodecyl sulfate sample buffer, and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis, along with selected samples from earlier steps in the procedure. Analysis of the fluorogram (not shown) in a scanning laser densitometer (LKB Instruments, Inc.) demonstrated that gp118 accounted for 77% of the total area under all detectable bands in the KSCN eluate.

To further improve upon the percentage of radiochemical purification, we tested a three-step chromatographic procedure which included serial Affi-Gel blue, *L. culinaris*-Sepharose 4B, and immunoadsorbent columns. A starting sample containing 7.7×10^6 cpm of [^{35}S]methionine was applied to an Affi-Gel blue column; unretarded protein in the effluent was collected and applied onto the lentil-Sepharose column. Fractions after elution with 0.5 M α-MM were combined, reduced in volume, and subsequently applied onto the immunoadsorbent (anti-gp118) column. After desorption with 3 M KSCN, the entire eluate along with

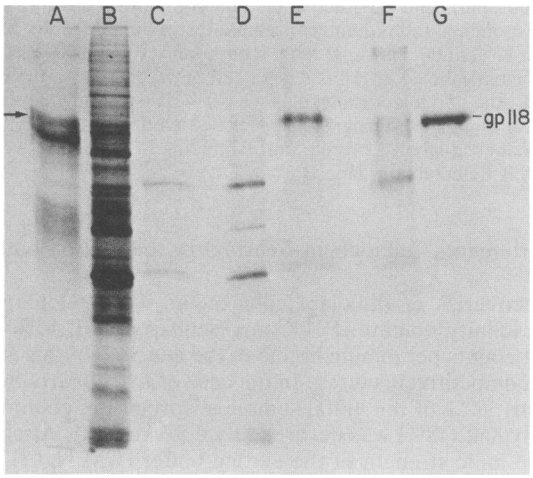

FIG. 4. Fractionation of detergent-solubilized VZV extract by serial affinity chromatography on immobilized Cibacron blue, lentil lectin, and immunoadsorbents. A sample of Nonidet-solubilized [^{35}S]methionine-labeled VZV-infected cell extract (7.7×10^6 cpm) was adsorbed onto an Affi-Gel blue column for 20 min. Unbound material which represented 22% of the original sample was applied onto the lentil lectin-Sepharose column, and further fractionation was carried out on an immunoaffinity column. Lane A, Nonidet-solubilized [^{14}C]glucosamine-labeled VZV-infected cell extract (20,000 cpm), the location of gp118 is designated by an arrow; lane B, Nonidet-solubilized [^{35}S]methionine-labeled VZV-infected cell extract, which was the initial antigen preparation of serial chromatography (40,000 cpm); lane C, proteins unretarded by Cibacron blue-Sepharose (6,800 cpm); lane D, proteins unretarded by immobilized *L. culinaris* (8,600 cpm); lane E, proteins eluted from lentil lectin-Sepharose with α-MM (7,000 cpm); lane F, effluent from the immunoaffinity column (10,000 cpm); lane G, proteins eluted from immobilized monoclonal antibody with 3 M KSCN (7,500 cpm). The location of gp118 is designated in the margin to the right of lane G in the 10% acrylamide–*N*-*N*′-diallytartardiamide slab gel.

TABLE 1. Chromatographic purification of VZV glycoprotein gp118

Step	Total protein (μg)	[^{35}S]methionine (cpm)[a]	Sp act ([^{35}S]methionine per μg)	% Recovery	Fold purification
Initial VZV extract	200.0	5.5×10^4	2.8×10^2	100	1
Final eluate	0.19	7.5×10^3	3.8×10^4	13.6	1,025

[a] In the densitometrogram of lane B in Fig. 4, the gp118 peak represented 0.72% of the 7.7×10^6 cpm in the initial [^{35}S]methionine-labeled sample.

samples from each previous step were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Fig. 4). When the chromatograms from the two experiments were compared, it was evident that the number and amount of additional proteins in the specific eluates had been further reduced by the three-step rather than the two-step chromatographic purification. This visual impression was confirmed by densitometric scanning of the fluorogram, which demonstrated that the gp118 peak represented in lane G of Fig. 4 included nearly 99% of the area under the tracing. On the basis of the total protein in the initial sample and the final eluate and the percentage of the label estimated to be associated with gp118, we calculated a fold purification of 1,025 after the three-step chromatographic procedure (Table 1).

Lectin chromatography has further defined the chemical nature of gp118. The saccharide-binding site of the well-characterized phytohemagglutinin ConA is most complementary to α-D-mannopyranosyl residues (6, 18). Since gp118 bound to this lectin and was efficiently eluted with the competitive inhibitor α-MM, it is presumably an asparagine-linked carbohydrate with a common core containing three or more mannose units. It is unlikely to include large neutral oligosaccharides with seven to nine mannose residues because these sugars are difficult to elute from ConA with α-MM (14). Furthermore, glycoproteins binding to ConA-Sepharose tend not to be tri- and tetraantennary complex types but usually are acidic biantennary complex, high-mannose, or hybrid-type oligosaccharides (4, 14, 16). The viral glycoprotein also was retained by lentil lectin, which binds to the same mannosyl residues as ConA but with other structural and compositional constraints, e.g., Kornfeld et al. (16) have demonstrated that attachment of a glycopeptide to lentil, but not to ConA, is dependent on the presence of a fucose molecule on the innermost *N*-acetylglucosamine residue. Based on its lectin affinities as well as its incorporation of both tritiated mannose and fucose, the VZV glycoprotein gp118 appears to contain predominantly acidic asparagine-linked, biantennary complex-type, or hybrid-type fucosylated oligosaccharides.

Cummings and Kornfeld (4) recently presented a detailed schema for the resolution of *N*-glycosidically linked murine cell surface glycoproteins by serial affinity chromatography on immobilized lectins, and Hunt (14) defined lectin affinity chromatographic procedures for characterization of Rous sarcoma virus oligosaccharides. As previously mentioned, Eisenberg et al. (5) reported that herpes simplex virus glycoprotein gD could be purified to homogeneity from a detergent-solubilized cytoplasmic extract by five sequential immunoaffinity chromatograms. Our results with VZV gp118 clearly demonstrate that preliminary chromatographic steps with either lectin or other ligands, such as blue dye or heparin, will obviate the need for repeated applications of the specific eluate to a monoclonal antibody-Sepharose column. We conclude, therefore, that fractionation of individual VZV glycoproteins by serial affinity chromatography is a specific and reproducible procedure which not only yields a highly purified product but also provides important information about the oligosaccharide moieties of the isolated glycoprotein.

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