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Membrane Proteins Specified by Herpes Simplex Viruses

IV. Conformation of the Virion Glycoprotein Designated VP7(B_2)

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The herpes simplex virus glycoprotein designated VP7(B_2) is extracted from virions by nonionic detergent in the form of an oligomer, whereas the other detergent-soluble envelope proteins appear to be extracted as monomers. The subunits of the VP7(B_2) oligomer cannot be dissociated by 2-mercaptoethanol and are also resistant to dissociation by a mixture of sodium dodecyl sulfate and 2-mercaptoethanol, except at elevated temperature. The oligomeric form of solubilized VP7(B_2) appears to be predominantly dimeric, based on the sedimentation rates in sucrose gradients and the electrophoretic mobilities in sodium dodecyl sulfate-containing acrylamide gels of the undissociated and heat-dissociated forms of VP7(B_2).

The virion of herpes simplex virus (HSV) is composed of DNA, lipids, and approximately 25 to 30 proteins, including five or six glycoproteins (9, 22, 24, 30). Most of these structural proteins are acquired during the process of envelopment (7, 30), as the nucleocapsid buds through modified patches of the inner nuclear membrane (3, 17, 18). The envelope has several morphologically identifiable components: a lipid-containing membrane; small spikes that project from the outer surface of the membrane; and an amorphous mass of electron-dense material that occupies space between the capsid surface and lipid bilayer (reviewed in reference 24). Although the organization of proteins in the envelope is not well understood, the glycoproteins appear to be exposed at the surface of the virion, and the non-glycosylated proteins are apparently located inside the lipid bilayer (20; reviewed in reference 24 and P. G. Spear, *in* H. A. Blough and J. M. Tiffany, *Cell Membranes and Viral Envelopes,* in press).

A major function of the HSV envelope, and of the envelope glycoproteins in particular, is to promote the infective entry of virus into a host cell. Previous studies (1, 9, 29) have demonstrated that HSV type 1 (HSV-1) virions contain at least four major glycoproteins and several minor glycosylated species, some of which appear to be partially glycosylated forms of the four major glycoproteins. Results presented in the accompanying paper (26) provide evidence that one of the envelope glycoproteins, designated VP7(B_2), plays a role in promoting the penetration of virus into host cells, but is apparently not required for the adsorption of virus to cells. The individual functions of the other envelope proteins and glycoproteins have not yet been identified.

The studies reported here were focused on investigating some physical properties of detergent-solubilized HSV-1 envelope proteins and on characterizing specific aspects of the structure of VP7(B_2). Sedimentation and electrophoretic analyses of the HSV-1 envelope proteins were performed, and an oligomeric form of glycoprotein VP7(B_2) was identified and partially characterized.

MATERIALS AND METHODS

Cells and viruses. African green monkey cells (Vero) and HEp-2 cells were grown as monolayer cultures in Dulbecco-modified Eagle minimal essential medium supplemented with 10% fetal calf serum (both obtained from K. C. Biologicals, Inc., Lenexa, Kans.). The virus strains used were HSV-1[F], which has been passaged a limited number of times in HEp-2 cells at low multiplicity and whose properties have been described (6, 7, 9, 13, 24, 29, 30), and the polykaryocyte-forming mutant designated HSV-1[MP] (6, 10, 13, 15, 23).

Radioactive precursors and other reagents used. New England Nuclear Corp. (Boston, Mass.) was the supplier of L-[^{35}S]methionine (100 to 400 Ci/mmol) and D-[1-^{14}C]glucosamine hydrochloride (45 to 55 mCi/mmol). Proteins used as standards in the rate zonal sedimentation analyses were human hemoglobin (type IV) and human gamma globulin (Cohn fraction II), both obtained from Sigma Chemical Co. (St. Louis, Mo.). The proteins used for molecular weight standards in acrylamide gel electrophoresis were myosin, a gift from D. Fischman (New York University); bovine thyroglobulin and bovine serum

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albumin, both obtained from Sigma Chemical Co.; and beta-galactosidase (*Escherichia coli*) obtained from Worthington Biochemical Corp. (Freehold, N.J.). The chemicals used for acrylamide gel electrophoresis were purchased from Bio-Rad Laboratories (Richmond, Calif.). The nonionic detergent Nonidet P-40 (NP-40) was obtained from Gallard Schlesinger Mfg. Corp. (Carle Place, N.Y.), and Dextran T10 is a product of Pharmacia Fine Chemicals AB (Uppsala, Sweden).

Purification of virions. Virions were purified from cytoplasmic extracts of infected cells essentially as described by Spear and Roizman (30) and modified by Heine et al. (9) and Cassai et al. (1). Briefly, HEp-2 cell monolayers in roller bottles were infected with virus at a multiplicity of 3 PFU/cell and were maintained in medium 199 supplemented with 1% fetal calf serum (199-V) for 72 h at 32°C. Radioactive precursors were added to the cells at 5 h after infection either in 199-V containing ½ the usual concentration of glucose and [^{14}C]glucosamine at 0.75 μCi/ml or in 199-V containing 1/10 the usual concentration of methionine and [^{35}S]methionine at 1 μCi/ml. Details on the purification of virions are given in the accompanying paper (26).

Extraction of virion envelope proteins. Portions of the Dextran T10 solution, containing purified virions from 4×10^8 infected cells (approximately 0.25 mg of protein), were placed into tubes for the SW27 rotor and diluted at least 1:4 with 1 mM phosphate buffer (pH 8.0), and the virions were collected by centrifugation at 25,000 rpm for 2 h (1.4×10^7 *g*-min). Each virion pellet was then suspended in 1.0 ml of a solution containing 0.5% NP-40 and 0.15 M NaCl in 1 mM phosphate buffer (pH 8.0), and the suspensions were placed on ice for 10 min. In some experiments either 5% 2-mercaptoethanol, 0.01 M dithiothreitol, or both were included in the extraction solution. Extracted proteins were separated from the insoluble residue by centrifugation of the suspensions at 25,000 rpm and 5°C for 2 h in the SW27.1 rotor, followed by careful aspiration of the supernatant fractions.

Centrifugation of the solubilized envelope proteins in sucrose gradients. Samples (0.5 ml) of the supernatants obtained by NP-40 extraction of virions were layered over 5 to 30% (wt/vol) sucrose gradients prepared in the extraction buffer (and formed in polyallomer tubes), and the gradients were centrifuged for 24 h at 5°C and 35,000 rpm in the SW41 rotor (3.0×10^8 *g*-min). After centrifugation, each gradient was fractionated by insertion of a cannula to the bottom of the tube followed by aspiration of the contents through the cannula with the aid of a peristaltic pump; fractions of 1 ml were collected. Samples of each fraction were taken for quantitation of trichloroacetic acid-precipitable radioactivity (25 μl) and also for sodium dodecyl sulfate (SDS)-acrylamide gel electrophoresis (100 μl), which was performed as described by Heine et al. (9) and in the accompanying paper (26).

Centrifugation in sucrose gradients was also performed under conditions designed to determine whether detergent-solubilized proteins from virions could float from a position at the bottom of the gradient. A supernatant containing proteins extracted from virions as described above was made 40% with respect to sucrose and placed in the bottom of a polyallomer tube for the SW41 rotor. A gradient of 5 to 30% (wt/vol) sucrose, prepared in extraction buffer as above, was layered on top of the sample. The tubes were then centrifuged and fractionated as described above.

Electrophoresis of proteins in polyacrylamide gels containing SDS. The methods used were as described in the accompanying paper (26). Samples of NP-40 extracts from virions or of fractions from gradients were prepared for electrophoresis by the addition of 4×-concentrated dissociation buffer to yield concentrations of the components as follows: 0.05M Tris-hydrochloride (pH 7.0), 5% 2-mercaptoethanol, 2% SDS, and a trace of bromophenol blue. The NP-40-insoluble residue from virions was dissolved directly in the dissociation buffer. Except where noted otherwise, the samples were heated before electrophoresis by placement in a boiling water bath for 2 min.

RESULTS

Extraction of envelope proteins from HSV-1 virions. Our efforts to characterize intermolecular associations among virion envelope proteins were focused on analyses of these proteins after their extraction from virions with the nonionic detergent NP-40 and after their separation from the insoluble residue by centrifugation. Relatively mild conditions were used for extraction (0.5% NP-40 and 0.15 M NaCl in 1 mM phosphate buffer, pH 8.0), as described in Materials and Methods, to favor conservation of native conformation of the solubilized proteins. The virions used were purified from HEp-2 cells infected either with HSV-1[F], a wild-type virus strain whose properties have been extensively studied (reviewed in ref. 24), or with HSV-1[MP], a fusion-inducing mutant (10, 23) that fails to produce one of the HSV-1 glycoproteins (9, 13, 15).

The electropherogram presented in Fig. 1 shows which of the HSV-1[F] virion proteins were extracted under the solubilization conditions used. As was previously reported (7, 30), NP-40 is highly selective in its action and extracts primarily the glycosylated virion proteins, the major species of which are here designated VP8(C_2), VP7(B_2), VP8.5(A), and VP18(D_2). At least three non-glycosylated virion proteins are also partially solubilized by NP-40, including VP7.5, which comigrates with glycoprotein VP7(B_2), VP16, and traces of VP21. Nucleocapsid structure is preserved under these conditions of extraction, as was previously shown (7, 30).

It should be noted that the virion glycoproteins were not quantitatively extracted by NP-40 under the conditions used here (only 60% of glucosamine-labeled proteins were recovered in the supernatant fractions), although all species appeared to be represented in the detergent-

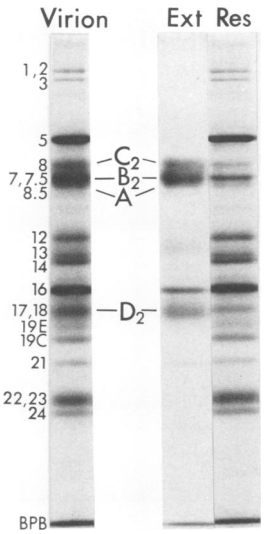

FIG. 1. *Electropherogram of [^{35}S]methionine-labeled polypeptides from intact HSV-1[F] virions, from an NP-40 extract of virions (Ext), and from the NP-40-insoluble residue (Res). The polypeptide bands are numbered according to the system proposed by Spear and Roizman (30) and Heine et al. (9), and the major glycoproteins were also assigned alphabetic designations by Spear (29).*

solubilized supernatant fractions. Elevation of the NP-40 concentration did not improve extractability of the glycoproteins, whereas increases in the NaCl concentration or pH did (unpublished data). We chose, however, to use the more moderate conditions described here (0.15 M NaCl and pH 8.0) because extremes of pH and high salt concentrations are known to dissociate the subunits of certain oligomeric proteins. Inclusion of reducing agents in the extraction buffer (5% 2-mercaptoethanol or 0.01 M dithiothreitol) increased the percentage of glucosamine-labeled proteins found in the supernatant fractions to approximately 80%, but did not alter the relative quantities of each species extracted (data not shown).

Rate zonal sedimentation of the NP-40-soluble virion envelope proteins in the presence or absence of reducing agents. This series of experiments was designed to determine the relative sedimentation rates in sucrose gradients of individual proteins in the NP-40 extracts and also to determine whether sedimentation of any of the envelope proteins could be significantly altered by the presence of reducing agents. HSV-1[F] virions were extracted as described in Materials and Methods with the NP-40-containing buffer, which included also (i) 5% 2-mercaptoethanol, (ii) 0.01 M dithiothreitol, (iii) both, or (iv) neither reducing agent. Aliquots of the supernatants obtained after removal of the insoluble residue were layered on 5 to 30% sucrose gradients prepared in the same solution that was used for extraction, and after centrifugation the gradients were fractionated and samples were removed from each fraction for estimation of trichloroacetic acid-precipitable radioactivity and for analysis by electrophoresis on SDS-acrylamide gels. The autoradiograms presented in Fig. 2 show the final distribution of each envelope polypeptide (labeled either with [^{35}S]methionine or with [^{14}C]glucosamine) in the gradients and are representative of results obtained under all the different conditions described above. The marker proteins (hemoglobin and γ-globulin), whose final positions after centrifugation are marked with arrows, were analyzed simultaneously on separate gradients in the absence of reducing agents; their sedimentation rates were the same in the presence or absence of NP-40.

Because the distributions of trichloroacetic acid-precipitable radioactivity and of individual polypeptides were the same whether or not reducing agents were used during extraction and sedimentation, we can conclude that the solubilized envelope proteins were not engaged in intermolecular associations mediated by disulfide bonds alone. Only the results obtained in the presence of 2-mercaptoethanol are shown in Fig. 2, and these conditions were chosen for all other experiments to be described because inclusion of the reducing agent significantly, but nonselectively, enhanced extraction of the glycoproteins from virions.

The results presented in Fig. 2 demonstrate a unimodal distribution of each envelope protein in the gradients, with the possible exception of glycoprotein VP7(B_2). Because VP7(B_2) is difficult to resolve from VP8(C_2) and VP8.5(A) by electrophoresis on SDS-acrylamide gels, there was some uncertainty as to whether any VP7(B_2) was present in fractions 9, 10, and 11 of the sucrose gradients. In an attempt to resolve this uncertainty, sedimentation analyses similar to the ones presented in Fig. 2 were done with envelope proteins extracted from HSV-1[MP] virions. Because HSV-1[MP] fails to produce VP8(C_2) (9, 13, 15) and because its VP7(B_2) and VP8.5(A) are more readily resolved by electrophoresis, it was possible to demonstrate that glycoprotein VP7(B_2) of HSV-1[MP] had a unimodal distribution in the sucrose gradients (Fig. 3) and sedimented to approximately the same position (fractions 6 and 7) as that occupied by the identifiable peak of VP7(B_2) specified by HSV-1[F] (compare Fig. 2 and 3). In fact, the sedimentation patterns of all the envelope proteins specified by these two HSV-1 strains,

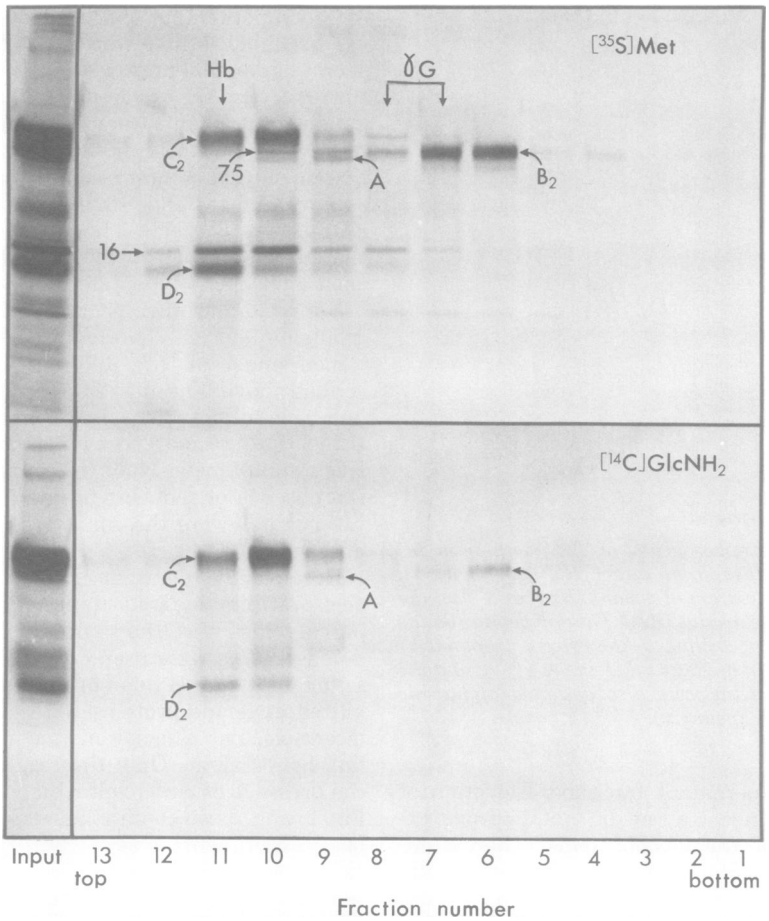

FIG. 2. *Electropherograms of HSV-1[F] envelope proteins from fractions of sucrose gradients. Also shown are the electrophoretic profiles of proteins present in samples layered on the gradients (input). In this particular experiment, 2-mercaptoethanol was included in the NP-40 extraction buffer and in the gradients, but similar results were obtained with 0.01 M dithiothreitol or without the reducing agents. The arrows mark the positions of human hemoglobin (Hb) and human γ-globulin (γG) standards, which were analyzed simultaneously under nonreducing conditions on separate sucrose gradients.*

which are unrelated with respect to origin, were remarkably similar, with the major exception that the species designated VP8(C_2) was absent from the gradients of the HSV-1[MP] proteins, consistent with the previously mentioned reports.

Certain other features of the sedimentation analyses shown in Fig. 2 and 3 should be noted. (i) A [^{35}S]methionine-labeled band [whose electrophoretic mobility is faster than that of VP7(B_2)] was sometimes detected in the fractions that contained VP7(B_2) (Fig. 3). Its relative mobility is similar to that of an under-glycosylated intermediate in the processing of VP7(B_2) (29); this intermediate, designated B_1, accumulates in the internal membranes of infected cells (E. M. Schechter and P. G. Spear, Abstr. Annu. Meet. Am. Soc. Microbiol. 1975, S206, p. 248) and is sometimes detected in preparations of purified virions. The sedimentation rate of VP7(B_2) does not depend on its presence, however (compare Fig. 2 and 3). (ii) A non-glycosylated or poorly glycosylated polypeptide designated VP7.5, whose electrophoretic mobility is identical to that of VP7(B_2), sediments more slowly than VP7(B_2) in sucrose gradients; although VP7.5 is clearly differentiable from VP7(B_2) by this criterion, we do not know whether these proteins are related. (iii) The glycoprotein designated VP8(C_2) was present in three fractions (9, 10, and 11) of the sucrose gradients shown in Fig. 2, and it was observed that the material in fraction 11 had a somewhat greater electrophoretic mobility in SDS gels

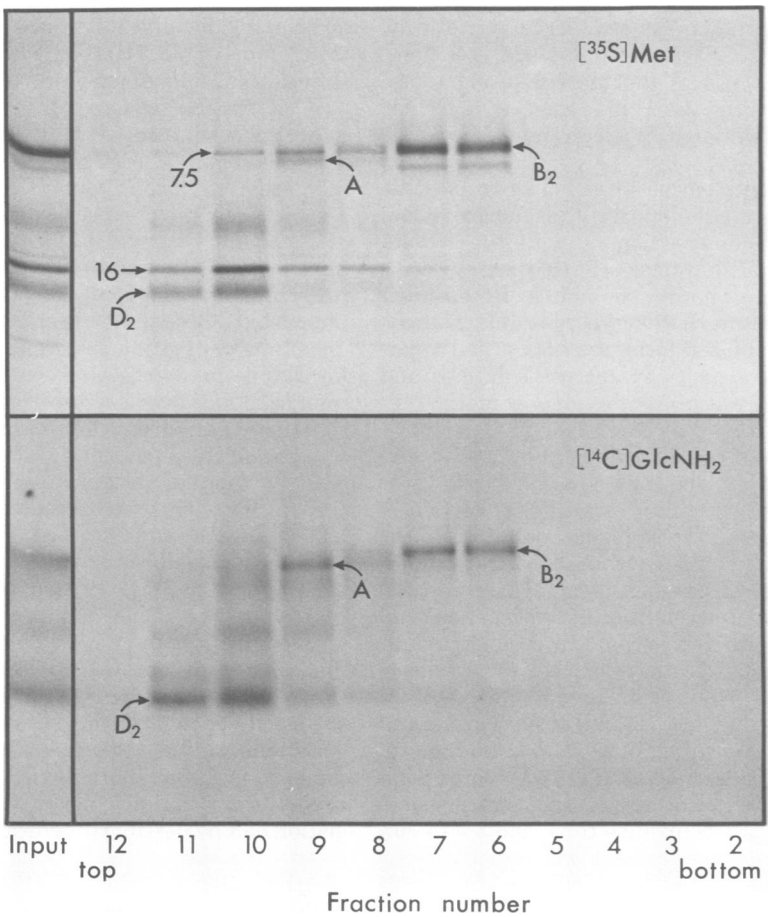

FIG. 3. *Electropherograms of HSV-1[MP] envelope proteins from fractions of sucrose gradients. Also shown are the electrophoretic profiles of proteins present in the samples layered on the gradients (input). The extracts were prepared and centrifuged on gradients as described in the legend to Fig. 2 and the text.*

than did the material in fraction 9. We suspect that heterogeneity in glycosylation affects both the sedimentation rate and the electrophoretic mobility of VP8(C_2); we cannot, however, rule out the possibility that VP8(C_2) actually consists of more than one polypeptide, although HSV-1[MP] fails to produce any of the material designated VP8(C_2).

Both sedimentation analysis in sucrose gradients and electrophoretic analysis on SDS-acrylamide gels can yield estimates of molecular weight under appropriate conditions. In the experiments reported here, electrophoresis in the presence of SDS and 2-mercaptoethanol should provide information about the denatured and fully dissociated polypeptides, whereas the sedimentation analyses in the presence of NP-40 could reveal the presence of oligomeric forms of the envelope proteins. It is recognized that estimations of the molecular weights of membrane glycoproteins present special problems with either of these techniques, due at least in part to unknown effects of their interactions with detergents and to the presence of carbohydrate side chains. Notwithstanding these considerations, comparisons of the relative sedimentation rates of the envelope proteins in sucrose gradients with their electrophoretic mobilities in SDS-acrylamide gels and with the sedimentation rates of marker proteins (hemoglobin and γ-globulin) revealed certain anomalies. The sedimentation of VP8(C_2), relative to those of hemoglobin (64,500 [4]), γ-globulin (150,000 [4]), and other HSV-1 envelope proteins, seems too slow to be consistent with its apparent monomeric molecular weight previously estimated by SDS-acrylamide gel electrophoresis (Table 1); the same kinds of considerations suggest that the sedimentation of VP7(B_2) is anomalously fast for the monomeric form of this polypeptide.

On the other hand, the sedimentation rates of VP7.5, VP16, VP8.5(A), and VP18(D_2), compared with those of the marker proteins, are consistent with their monomeric molecular weights as estimated by electrophoresis (Table 1).

These results led us to explore two possible explanations for the apparent discrepancies between the sedimentation rates of the NP-40-solubilized glycoproteins VP7(B_2) and VP8(C_2) and the electrophoretic mobilities of their monomeric forms on SDS-acrylamide gels: namely, that the solubilized form of VP8(C_2) had a particularly low density in sucrose solutions and that VP7(B_2) was present as an oligomer in NP-40 solution.

Analysis of the NP-40-soluble HSV-1 virion envelope proteins on flotation gradients. The possibility existed that certain of the anomalies in the sedimentation rates of the glycoproteins could have resulted from differences in the relative densities of these proteins due to differential retention of lipid and/or binding of detergent. If this were the case, then some of the extracted proteins, placed in the bottom of a sucrose gradient similar to the one used for rate zonal sedimentation, might float in the gradient during centrifugation. To test this hypothesis, an NP-40 extract of HSV-1[F] virions was made 40% with respect to sucrose and placed in the bottom of a centrifuge tube, and a 5 to 30% continuous sucrose gradient was formed directly on top of the sample. After centrifugation for 24 h at 3×10^8 *g*-min, it was found that essentially all the envelope proteins (labeled either with [^{35}S]methionine or with [^{14}C]glucosamine) had remained at the bottom of the gradients in the 40% layer. Approximately 80 to 85% of the input radioactivity was recovered in the first (bottom) fraction and 12 to 18% in the second fraction. It would appear, therefore, that the densities of all the solubilized envelope proteins are not significantly less than that of 40% sucrose and that differences in their densities probably do not account for the large differences in the sedimentation rates of VP7(B_2) and VP8(C_2) (Fig. 2).

TABLE 1. *Apparent molecular weights of HSV-1 envelope proteins*

HSV-1 polypeptide	Apparent mol wt[a] ($\times 10^3$)
VP8(C_2)	129
VP7(B_2)	126
VP7.5	126
VP8.5(A)	119
VP16	68
VP18(D_2)	59–62

[a] Estimated from the electrophoretic mobilities of the SDS-dissociated polypeptides in diallyltartardiamide-cross-linked acrylamide gels (9).

Sedimentation and electrophoretic analysis of the NP-40-soluble virion envelope proteins with and without heat treatment. The possibility also existed that the fast sedimentation of glycoprotein VP7(B_2) was due to association of the polypeptide chains in the form of oligomers, the subunits of which would then have been dissociated by the treatment used to prepare samples for SDS-acrylamide gel electrophoresis. To determine the effects of eliminating one of the denaturing procedures routinely used for sample preparation (heating to 100°C for 2 min), fractions from sucrose gradients of NP-40-solubilized virion envelope proteins were analyzed by electrophoresis on SDS-acrylamide gels without prior heat treatment of the samples. All the envelope proteins, except for glycoprotein VP7(B_2), migrated to the same positions as seen when heat denaturation was employed prior to electrophoresis (Fig. 4; compare with Fig. 2). Most of the radiolabeled material that forms the fast-sedimenting peak and which was previously identified as glycoprotein VP7(B_2) migrated very slowly, although some traces were detectable at the usual position. This result suggests that glycoprotein VP7(B_2) is present in the form of a dimer or multimer, both in NP-40 solutions and in SDS solutions, and that this oligomeric association can be destroyed by heat.

An experiment was done to determine whether glycoprotein VP7(B_2), treated with both NP-40 and SDS, would sediment at the same rate in sucrose gradients as NP-40-solubilized VP7(B_2) and whether heat denaturation could alter its sedimentation rate. An NP-40 extract from [^{14}C]glucosamine-labeled HSV-1[F] virions was fractionated on a sucrose gradient; the fraction containing isolated glycoprotein VP7(B_2) was then diluted by the addition of sample buffer for SDS-electrophoresis. One sample was heated in a boiling water bath for 2 min, and another sample was not heated. Both samples were analyzed on sucrose gradients under conditions similar to those used in the previous experiments. The distributions of trichloroacetic acid-precipitable radioactivity are shown in Fig. 5. Radiolabeled protein in the unheated sample formed two peaks in the gradient: a fast-sedimenting peak, at the position previously seen for glycoprotein VP7(B_2) in NP-40 solution, and another peak sedimenting near the top of the gradient (compare Fig. 2 and 5). On the other hand, radiolabeled protein in the heated sample formed only one peak in the gradient, at the same position as the smaller, slow-sedimenting peak just described. Electro-

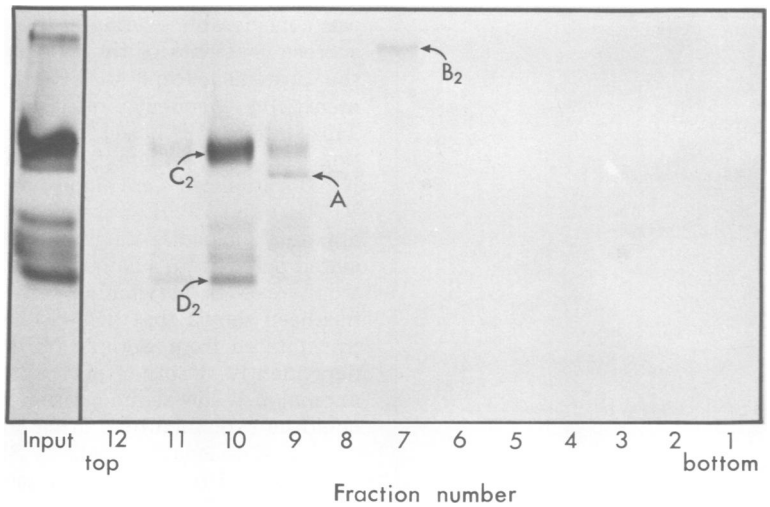

FIG. 4. *Electropherogram of [^{14}C]glucosamine-labeled HSV-1[F] envelope proteins in fractions from a sucrose gradient. The samples were not heated before electrophoresis, in contrast to the usual procedure.*

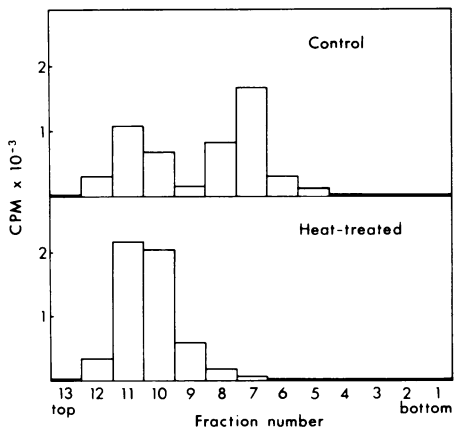

FIG. 5. *Rate zonal sedimentation of purified glycoprotein VP7(B_2) obtained from HSV-1[F] virions. The fraction containing glycoprotein VP7(B_2), from a sucrose gradient similar to those shown in Fig. 2, was mixed with ⅓ volume of concentrated sample buffer for SDS-acrylamide gel electrophoresis and was divided into two aliquots, only one of which was heated by submersion in a boiling water bath for 2 min. Each aliquot was layered over a 5 to 30% (wt/vol) sucrose gradient prepared in 1 mM phosphate buffer (pH 8.0) containing 0.5% NP-40, 0.15 M NaCl, and 5% 2-mercaptoethanol; the gradients were centrifuged and analyzed as described in the text.*

phoretic analysis of the gradient fractions (without heat treatment of the samples) revealed that the slow-sedimenting peak in both gradients contained the familiar monomeric glycoprotein VP7(B_2), whereas the fast-sedimenting peak contained the high-molecular-weight protein seen in Fig. 4 and only traces of the smaller component (data not shown). We can conclude that this high-molecular-weight protein is converted to monomeric VP7(B_2) by heating in the presence of SDS and 2-mercaptoethanol.

The apparent molecular weight of the fast-sedimenting oligomeric form of VP7(B_2) was estimated by co-electrophoresis with proteins of known molecular weight on SDS-acrylamide gels and was found to be about 250,000, as compared with about 126,000 for the heat-dissociated form of this glycoprotein (Fig. 6). Moreover, the oligomer sedimented in sucrose gradients approximately twice the distance as did the heat-dissociated form of VP7(B_2) under the conditions employed here (Fig. 5). These data suggest that the larger, fast-sedimenting protein is a dimer of the smaller component. It should be noted that VP7(B_2) could not be detected in the bottom fractions of the sucrose gradients, suggesting that the dimer of this glycoprotein is the predominant form present in the NP-40 extracts.

DISCUSSION

The oligomeric conformation of solubilized glycoprotein VP7(B_2) is maintained at least in part by noncovalent interactions, and the oligomer is apparently composed of two polypeptides with similar electrophoretic mobility, perhaps of two identical glycopolypeptides. These conclusions are based on the following observations. (i) The oligomer is relatively stable even in the presence of SDS and 2-mercaptoethanol, but it can be dissociated by heat (Fig. 5). (ii) Only one polypeptide band was usually detected by electrophoretic analysis of the oligomer isolated

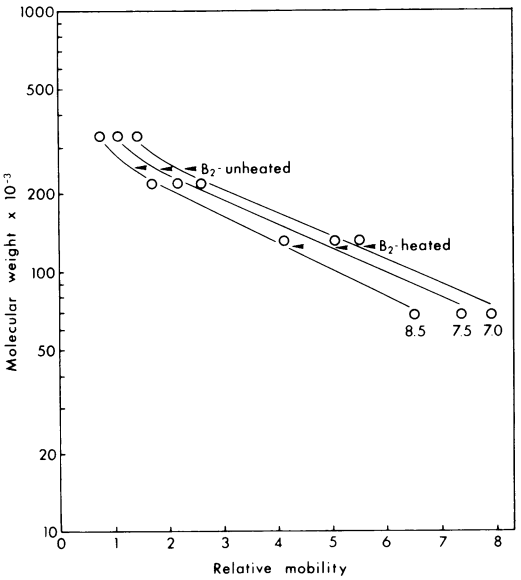

FIG. 6. *Apparent molecular weights of the two forms of glycoprotein VP7(B_2), as estimated by their electrophoretic mobilities in SDS-acrylamide gels relative to proteins of known molecular weight. The proteins used as standards and the molecular weights of their subunits are: thyroglobulin, 335,000 (5, 31); myosin, 220,000 (28, 35; L. C. Gershman and P. Dreizen, Biophys. J. 9:A235); beta-galactosidase, 130,000 (34); bovine serum albumin, 68,000 (33).*

from sucrose gradients, except for small amounts of a component that is probably a partially glycosylated form of VP7(B_2) (Fig. 2 and 3). (iii) The relative sedimentation rates (Fig. 5) and electrophoretic mobilities (Fig. 6) of the oligomer and heat-dissociated monomer are consistent with a dimeric conformation.

The dimeric conformation of glycoprotein VP7(B_2) is probably not an artifact of NP-40 solubilization because it can also be detected in extracts prepared directly from virions with SDS (26). Although the association of VP7(B_2) monomers could possibly be caused by the detergents or extraction conditions used, the possibility exists that VP7(B_2) may be present in virions as a dimer. One of the erythrocyte membrane glycoproteins can also be solubilized in the form of an SDS-stable dimer (16), and the biological activities of several glycoproteins, including interferon (32) and the influenza virus hemagglutinin (14), can be recovered after removal of SDS, indicating that certain glycoproteins are resistant to complete denaturation by this detergent.

We found no evidence for any other intermolecular associations among the NP-40-solubilized HSV-1 envelope proteins. Although there was considerable overlap in the distributions in sucrose gradients of the envelope proteins, all the proteins except VP7(B_2) may have sedimented independently of others as monomers. This statement is based in part on the observation that the sedimentation rates of all the NP40-solubilized envelope proteins, except VP7(B_2) and VP8(C_2), were consistent with the apparent molecular weights of their SDS-dissociated polypeptide chains, as estimated by electrophoresis on acrylamide gels. In addition, it has been shown that VP8(C_2) can be immunoprecipitated from isotonic NP-40 solutions independently of other polypeptides (29). The anomalously low sedimentation rate of VP8(C_2) could be explained by a large error in the estimation of molecular weight by SDS-gel electrophoresis and/or by unusual properties of the molecule (affinity for detergent, carbohydrate side chains) that might influence its sedimentation rate.

We cannot, of course, rule out the possibility that specific associations among proteins in the virion envelope were destroyed by NP-40 extraction. Such associations may depend in part on the integrity of the lipid bilayer or may simply be unstable under the particular conditions employed in our experiments. In fact, it seems more than likely that multiple specific interactions among the envelope proteins are important for virion morphogenesis and envelope function. Further investigations along these lines may require attempts to stabilize intermolecular interactions prior to extraction and analysis of the envelope proteins.

The relationship between the conformation of VP7(B_2) and its function will be the focus of future investigations. Studies with a temperature-sensitive mutant, designated HSV-1[HFEM]tsB5, have provided evidence that glycoprotein VP7(B_2) in the virion envelope plays a role in viral penetration of the host cell (26) and that, in membranes of infected cells, it also has a role in HSV-induced cell fusion (15), suggesting that VP7(B_2) may mediate or promote membrane fusion. The mutant mentioned above will be useful for correlating specific aspects of VP7(B_2) structure with its function because, as described in the accompanying paper (26), its VP7(B_2) produced at permissive temperature does not form the kind of SDS-stable dimer described in this report.

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