Multimeric Forms of Herpes Simplex Virus Type 2 Glycoproteins

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Herpes simplex virus type 2-specific glycoproteins present in detergent extracts of infected cells were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis under conditions designed to permit detection of multimeric forms of these glycoproteins. Two high-molecular-weight glycosylated species were detected when samples were disrupted at lower temperatures or in the absence of any reducing agents. One multimer having an apparent molecular weight of 275,000 was identified as a multimer of the gA or gB glycoprotein or both. The second glycoprotein, having a molecular weight of approximately 230,000, was identified as a multimeric form of the gC glycoprotein. These data indicate that the gC as well as the gA and gB glycoproteins of herpes simplex virus type 2 may exist in a multimeric form.

In many enveloped viruses, the linking together of more than one glycoprotein by disulfide bonds to form the surface structures of the virion envelope has been reported (5-8, 12, 13). Studies by Sarmiento and Spear (11) on herpes simplex virus type 1 (HSV-1) have shown that the gB glycoprotein (apparent molecular weight, 119,000) present in virions exists, at least in part, as a dimer ($[gAB]_2$; apparent molecular weight, 250,000). The oligomeric form of the gA and gB glycoproteins have also been shown to occur in the infected cell (4). To date, there have been no reports suggesting that the gC glycoprotein (apparent molecular weight, 130,000) of HSV-1 can exist in a dimeric form. Using conditions described in this communication, we have also been unable to detect any species representing a dimer of the HSV-1 gC glycoprotein (data not shown).

In comparing the HSV-1 glycoproteins with those specified by HSV-2, it has been shown that the gA and gB glycoproteins of HSV-1 and HSV-2 share many biochemical and immunological properties as well as mapping in a colinear fashion on the viral genomes (1-3, 9, 10). In contrast, the gC glycoproteins of HSV-1 and HSV-2 which exhibit noncolinear mapping on the viral genomes are quite distinct from one another in their biochemical and immunological properties despite their similar molecular weights (2, 3, 10). The focus of this report is on the finding that, in contrast to the HSV-1 gC glycoprotein, the HSV-2 gC glycoprotein may exist in a multimeric form within the infected cell.

HEp-2 cells were infected with HSV-2 strain 186, labeled with D-[1- 14 C]glucosamine (specific

activity, 61 mCi/mmol) at 4 h postinfection, and harvested at 24 h postinfection. Infected cells were then prepared for sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis by disruption in 0.05 M Tris-hydrochloride (pH 6.7) containing 1% SDS and 1% 2-mercaptoethanol (ME), 1% SDS and 0.05 M iodoacetamide (IA), or 1% SDS at 25 or 100°C for 2 min. The electrophoretic profile of HSV-2-infected cells treated under these various conditions is shown in Fig. 1. When infected cells were disrupted in SDS-ME (reduced), all of the glycosylated species present, with the exception of those comprising the major glycoprotein region, migrated more slowly than when the cells were treated with SDS or with SDS-IA (nonreduced). This difference in migration of the reduced versus the nonreduced glycoproteins was apparently independent of the temperature at which disruption was carried out (25 or 100°C). In addition, the difference in the electrophoretic mobility between reduced and nonreduced samples appeared to be reflected by each of the glycoproteins independent of their apparent molecular weights. Since reduction with ME has its greatest effect on the integrity of disulfide bonds, it is probable that the change in electrophoretic mobility of the glycoproteins upon reduction is due to disruption of intra- or interpolypeptide disulfide bonds or both. Since the change in electrophoretic mobility is small and consistent for all of the glycoproteins, it is improbable that this change is generated by disruption of interchain bonds, i.e., multimeric forms of the glycoproteins. It is thus more likely that this change is due to reduction of intrachain disulfide bonds.

Within the major glycoprotein region, the gA

FIG. 1. Analysis of HSV-2 glycoproteins under reducing and nonreducing conditions. HSV-2-infected HEP-2 cells were labeled at 4 to 24 h postinfection with [¹⁴C]glucosamine (2 μ Ci/ml) and disrupted in 0.05 M Tris buffer (pH 6.7) containing 2% SDS (SDS), 2% SDS and 0.05 M iodoacetamide (SDS/IA), or 2% SDS and 2% 2-mercaptoethanol (SDS/ME) at either room temperature (25°C) or at 100°C for 2 min. Samples were electrophoresed on 8.6% gels as previously described (1). The major glycoproteins (gA, gB, gC, and gD) are identified in both reduced and nonreduced samples. Glycoprotein bands labeled [gC]₂ and [gAB]₂ represent the multimeric forms of the gC and the gA and gB glycoproteins, respectively, which are described in this communication.

and gB glycoproteins exhibited a similar behavior in that a greater electrophoretic mobility of the gA and gB glycoproteins was observed under nonreduced conditions as compared with their migration on SDS-polyacrylamide gel electrophoresis when reduced with ME (see Fig. 2). In contrast, no such marked variation in migration of the gC glycoprotein was detected, suggesting an absence of intrachain disulfide bonds in this glycoprotein. Such a finding is, in fact, not surprising, since this glycoprotein represents an extremely highly glycosylated species which incorporates very little amino acid isotopes (3). Thus, it would appear less likely that this glycoprotein would form intrachain disulfide bonds based simply on the extremely low amino acid content of this glycoprotein.

In addition to the previously described glycoproteins discussed above, two additional glycosylated species having apparent molecular weights of approximately 275,000 and 230,000, were observed under various conditions (Fig. 1). The highest-molecular-weight species (275,000)

was observed only in unheated samples. A similar glycosylated species found in HSV-1-infected cells has been shown by Spear and co-workers (4, 11) to be a multimeric form of the gA and gB glycoproteins. When procedures described previously were used (1), isolation of this component from HSV-2-infected cells and gel analyses have directly shown that this species does dissociate into the gA and gB glycoproteins (data not shown). Thus, this 275,000-dalton species represents a dimer composed of the gA or gB glycoproteins or both.

The second high-molecular-weight species (230,000) was prominent in all samples which were not reduced and, to a lesser extent, in the unheated, SDS-ME-treated sample. This glycoprotein appeared to be sensitive to reduction by ME, especially at elevated temperatures. Of interest was the observation that, when this species was present in infected cell extracts, the amount of material migrating within the major glycoprotein region was reduced, as compared with the amount present when the high-molecular-weight species was absent (SDS-ME at 100°C). Quantitative analyses of densitometric scans of such samples confirmed that the isotopic content of the high-molecular-weight species plus the major glycoprotein region in nonreduced samples is essentially identical to the isotopic content of the major glycoprotein region of reduced samples (Table 1). These results imply that the high-molecular-weight species represents a multimeric form of one of the glycoproteins which comprises the major glycoprotein region. Since the great majority of radioactive glucosamine incorporated into the major glycoprotein region is present in the HSV-2 gC glycoprotein rather than in the gA and gB glycoproteins (3), it appeared that the glucosamine-labeled material of 230,000 daltons could represent an oligomer of the gC glycoprotein.

To examine the possibility that this species does indeed represent a multimeric form of the HSV-2 gC glycoprotein, we used antisera prepared to the gC and the gA and gB glycoproteins of HSV-2 (3) using procedures previously described for the preparation of antisera to the HSV-1 glycoproteins (1). HEP-2 cells were infected with HSV-2 and labeled with [¹⁴C]glucosamine from 4- to 24-h postinfection. An extract was prepared by solubilization of infected cells in 1% Triton X-100 for 30 min at 4°C in the presence of 0.05 M IA. After removal of particulate matter by centrifugation at 100,000 $\times$ g for 30 min, the soluble extract was used in immune precipitation assays, employing anti-gAB (reactive with both the gA and gB glycoproteins) and anti-gC sera. All reactions were carried out in duplicate, and the resulting precipitates were washed three times with phosphate-buffered sa-

FIG. 2. Immune precipitations of HSV-2 glycoproteins analyzed in the presence or absence of a reducing agent. HSV-2-infected HEP-2 cells were labeled with [^{14}C]glucosamine as described in the legend to Fig. 1, and a detergent extract was prepared which contained 0.05 M IA. Antisera prepared to the gC glycoprotein (Anti-gC), the gA and gB glycoproteins (Anti-gAB), and a normal rabbit serum (NRS) were used to immune precipitate from the detergent extract. Immune precipitates were then washed and solubilized in either SDS-IA (nonreduced) or SDS-ME (reduced). The detergent extract used as starting material was also disrupted in each of the two systems for comparison. Polypeptides present in immune precipitates solubilized in SDS-ME (■) and SDS-IA (●) are indicated.

line containing 0.1% Triton X-100 and 0.05 M IA. One each of the final precipitates was then solubilized in disruption buffer containing 2% SDS and 2% ME, whereas the duplicates were solubilized with 2% SDS and 0.05 M IA. All immune precipitates were then analyzed by SDS-polyacrylamide gel electrophoresis (Fig. 2).

As indicated in Fig. 2, neither the gC glycoprotein nor the 230,000-dalton species was precipitated by the anti-gAB serum. Immune pre-

cipitates obtained with the anti-gC serum, however, did contain the gC glycoprotein. In addition, the nonreduced sample also contained the 230,000-dalton species. As found with whole-cell lysates, densitometric analysis indicated that the amount of radiolabel present in the gC band of the reduced sample was nearly identical to the sum of radiolabel found in the gC and 230,000-dalton bands of the nonreduced sample (data not shown). These data thus demonstrate an antigenic relationship between the gC glycoprotein and the 230,000-dalton species as well as the dissociation of the high-molecular-weight species into a form which comigrates with the gC monomer. Therefore, one may conclude that the gC glycoprotein of HSV-2 exists, at least partially, as an oligomer of itself, most probably being a dimer, based on the apparent molecular weight.

If it is assumed that the 230,000 multimer does indeed represent a dimer of the gC glycoprotein (approximate molecular weight, 130,000), it is interesting that the 230,000 molecular-weight estimate is considerably less than the molecular weight which would be expected (approximately 260,000), especially in light of the higher apparent molecular weight of the [gAB]₂ dimer (275,000), which is comprised of a glycoprotein(s) having a lower apparent molecular weight

TABLE 1. Densitometric quantitation of the gC glycoprotein and the gC dimer [gC]₂

Expt	Conditions	AUC (%) ^a	
		gC	[gC] ₂
1	SDS-ME, 100°C	1.00 (100.0)	
	SDS-IA, 100°C	0.82 (73.9)	0.29 (26.1)
2	SDS-ME, 100°C	1.00 (100.0)	
	SDS-IA, 100°C	0.82 (78.1)	0.23 (21.9)

^a Numbers represent the areas under the curve (AUC) generated by densitometric scanning of gel autoradiographs. In each experiment, values are compared to that of the gC glycoprotein disrupted at 100°C in the presence of ME. Numbers in parentheses represent the percent of isotope present in each of the two glycoprotein species.

than the gC glycoprotein. It is possible that the anomalous migration of the gC dimer is due to the highly glycosylated nature of the gC glycoprotein.

In conclusion, it is not surprising that the gA and gB polypeptides of HSV-2 can exist in a dimeric form, since these glycoproteins are very similar in many of their properties to the gA and gB glycoproteins of HSV-1 for which such dimerization has been previously reported (2-4, 11). However, the existence of a dimer of the HSV-2 gC glycoprotein ($[gC]_2$) does not have any precedence in the HSV-1 system. This lack of similarity is compatible with the antigenic and biochemical distinctness of the HSV-1 and HSV-2 gC glycoproteins despite their superficial similarities to one another, i.e., their both being more highly glycosylated than either the gA or gB glycoproteins and of only slightly greater apparent molecular weights than the gB glycoprotein (3). The significance of the ability of the HSV-2 gC glycoprotein to exist in a multimeric state is at present unclear but does serve to further demonstrate the distinctness of the HSV-1 and HSV-2 gC glycoproteins.

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