JOURNAL OF VIROLOGY, Nov. 1986, p. 803–806
0022-538X/86/110803-04$02.00/0

Vol. 60, No. 2

Oligomerization of Herpes Simplex Virus Glycoprotein B

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Received 21 April 1986/Accepted 26 July 1986

Glycoprotein B (gB) specified by herpes simplex virus can be extracted from virions or infected cells in the form of detergent-stable, heat-dissociable oligomers. The composition of the oligomers and requirements for their formation were investigated. Evidence is presented that the faster-migrating forms of the oligomers are homodimers of gB. Dimerization was shown to occur within minutes of polypeptide synthesis and did not depend on glycosylation, the expression of other viral proteins, or virion morphogenesis. The multiple, electrophoretically distinct forms of gB dimers differ in extent or rate of N-linked oligosaccharide processing and also have other differences that influence electrophoretic mobility.

The herpes simplex virus (HSV) genome encodes several membrane glycoproteins that are constituents of infected cell membranes and the virion envelope (for a review, see reference 20). One of these, designated gB, can be extracted from virions (16) or infected cells (2) in the form of oligomers that are, in part, resistant to dissociation by sodium dodecyl sulfate (SDS) and 2-mercaptoethanol but are sensitive to dissociation by heat. gB is essential for virion infectivity, at least part of its function relating to penetration of HSV into the cell (7, 15). Compared with most of the others, this glycoprotein appears to be quite highly conserved within the *Herpesviridae* group, based on studies of antigenic cross-reactivity (1, 10, 18) and amino acid homology (12).

Because the oligomers containing gB are relatively stable under the standard conditions used in SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (provided the samples are not heated), these oligomers can be detected in unheated samples as slowly migrating bands that disappear after heating. Multiple forms of gB oligomers were detected in this and previous (2, 16) studies. The purpose of this study was to characterize more fully the oligomers containing gB with respect to composition and requirements for formation.

Oligomer composition. Although previously published data (16) suggested that the oligomers were likely to be composed of gB alone among viral polypeptides, the possibility that cell components formed a part of the oligomers was not explored. We therefore purified gB monomers and oligomers by affinity chromatography, isolated oligomeric forms, dissociated these forms by heat, and identified the components by silver staining of SDS-polyacrylamide gels.

HEp-2 cells were infected with a strain of HSV type 1, HSV-1(KOS) (obtained from P. Schaffer, Harvard Medical School, Cambridge, Mass.), and at 24 h after infection, an extract was prepared in 20 mM Tris hydrochloride (pH 7.4)–0.5% Nonidet P-40–0.5% sodium deoxycholate–1% aprotinin–1 mM phenylmethylsulfonyl fluoride. The extract was passed through a column of Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, N.J.) to which the gB-specific monoclonal antibody (MAb) II-105-2 (11) had been coupled. After the column was washed with 10 mM NH_4HCO_3 (pH 7.5)–150 mM NaCl–0.5% sodium deoxycholate and with 10 mM NH_4HCO_3 (pH 7.5)–0.5% sodium deoxycholate, gB was eluted with 3 M potassium thiocyanate (pH 7.1) and dialyzed against 10 mM NH_4HCO_3–0.01% SDS.

Coomassie blue-stained bands detected by electrophoresis of unheated (lane a) and heated (lane b) samples of the purified gB are shown in Fig. 1A. A significant fraction of the unheated purified material migrated as oligomers, despite the possibilities for dissociation during affinity chromatography. The two slowest-migrating bands (marked with dots) have apparent molecular masses of 220 and 240 kilodaltons (kDa), whereas the two faster-migrating bands are about 100 and 120 kDa, as is characteristic for monomeric gB. The two monomeric forms of gB differ in the extent of posttranslational processing (6, 17, 21). In the heated sample, the slowly migrating bands (including material of even greater molecular mass than 200 and 240 kDa) were absent, and proportionately greater amounts of monomeric gB were present.

The bands marked with dots in lanes a and b (Fig. 1A) were excised from the gel, and the protein was extracted, heated, and analyzed by SDS-PAGE on another gel (lanes c to h). Silver staining did not reveal the presence of any polypeptides other than gB.

The availability of a transformed cell line carrying the gB gene made it feasible to test whether gB oligomers can form in the absence of most other HSV gene products. The cell line was obtained by cotransfection of Vero cells with plasmids containing a selectable marker (9) and the HSV-1(F) gB gene (L. Claesson-Welsh, D. WuDunn, and P. Spear, manuscript in preparation). The viral DNA fragment used (map coordinates 0.35 to 0.38) contains partial or complete information for at least one gene other than the gB gene (4, 14). Although the transformed cell line does not constitutively express gB, supertransfection with the cloned gene for the HSV-1 regulatory gene ICP4 (5) induces transient expression of gB (13). Extracts prepared from the supertransfected cells were fractionated by SDS-PAGE, transferred to nitrocellulose, and probed with an anti-gB antiserum (Fig. 1B, lanes a and b); samples of purified gB from infected HEp-2 cells (lanes c and d) were also probed. Oligomer formation occurred in the transformed cells as it did in infected cells. There were differences in migration rates of the oligomers. These differences, not explored further, were possibly due to different treatment of samples (cell extract versus affinity purification), the use of different virus strains (F and KOS), or the processing of gB in different cell lines (transformed Vero cells versus infected

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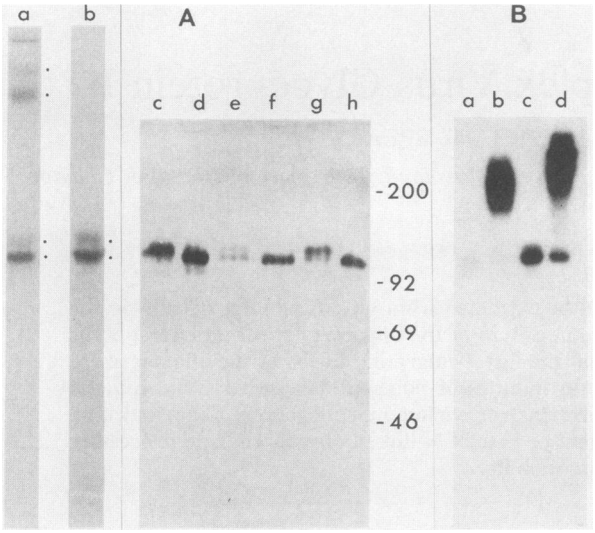

FIG. 1. Composition of gB oligomers. (A) Affinity-purified gB (20 μg) was either boiled (lane b) or not (lane a) before SDS-PAGE (3). Bands detected after staining with Coomassie blue (indicated by dots in lanes a and b) were cut out, and the material was extracted from the gel, boiled, and reloaded on a 10% SDS-polyacrylamide gel, which was silver stained after electrophoresis (lanes c to h). Lanes c to f contain material extracted from lane a, with lane c being a rerun of the highest-molecular-weight band. Lanes g and h are derived from lane b. (B) Western blot transfer analysis of extract from a gB-expressing transformed Vero cell line (lanes a and b) and affinity-purified gB (lanes c and d) by use of a rabbit anti-gB antiserum for detection. Samples in lanes a and c were boiled before analysis.

HEp-2 cells). The antiserum used reacts preferentially with oligomers.

The results described above provide evidence that the 220- and 240-kDa oligomers are composed principally, if not exclusively, of gB (unless one component is another polypeptide identical in electrophoretic mobility to gB). Moreover, a viral glycoprotein similar in size to gB, designated gH (8), could not be detected in oligomers by immunoprecipitation with the anti-gH MAb LP11 (8) (data not shown). Based on their apparent sizes, the 220- and 240-kDa oligomers are likely to be homodimers of gB.

Requirements for oligomer formation. The relationship between posttranslational processing of gB and oligomer formation was assessed by examining the properties of pulse-labeled forms of gB. HEp-2 cells infected with HSV-1(KOS) were labeled for 10 min with [^{35}S]methionine at 6 h after infection. Extracts were prepared for immunoprecipitation with an anti-gB MAb either immediately after the 10-min labeling period or after various periods of chase in nonradioactive medium. Heated and unheated samples of the immunoprecipitates were analyzed by electrophoresis (Fig. 2).

In the heated samples, a single form of gB (about 110 kDa) was detected after the pulse-label (Fig. 2, lane e). By 3 h of chase (lane g), the 120-kDa form of gB monomers was also present in significant amounts, although it is not well resolved in this autoradiogram. As has been observed previously (19), the amount of labeled precipitable gB increased steadily until at least 3 h of chase, possibly due to the relative insolubility of newly synthesized gB, and stable species of both 110 and 120 kDa accumulated.

In the unheated samples, oligomeric forms were evident immediately after the pulse-label. The percentage of total labeled gB detected as oligomers increased slightly but steadily during the chase, ranging from 35% at 30 min of chase to about 50% at 18 h. (With the use of greatly reduced concentrations of SDS [0.01%] in sample buffer, gel, and running buffer, most of the gB that accumulated was in oligomeric form [data not shown].) Multiple oligomeric species were detected in the 200- to 300-kDa range. The relative amounts of these various species did not change drastically during the chase. Moreover, the oligomers did not seem to be preferentially recruited from either of the gB monomer bands, as heating did not change the relative amounts of the two.

The compositions of the oligomeric species detected after a 10-min pulse-label and after a 3-h chase were determined. Labeled bands were excised from a gel, and the protein was extracted, dissociated by heat, and analyzed by SDS-PAGE (Fig. 3). As expected, after the 10-min pulse, all the oligomeric forms of gB (bands labeled a, b, and c) contained only the 110-kDa monomer. The bands labeled d and e were often coprecipitated from extracts of pulse-labeled cells but not after a chase. Their migration rates were not altered by heating. After the pulse-label and 3-h chase, complexes of gB that failed to penetrate the main gel (labeled f and possibly composed of dimer aggregates or other higher-order structures) contained both the 110- and 120-kDa species, whereas the resolvable oligomers were composed only of one or the other monomer form. Specifically, the bands labeled g, h, and i contained only the 120-kDa form, and the band labeled j (220 kDa) contained only the 110-kDa form.

The N-linked oligosaccharides on the 110-kDa monomer of gB were all sensitive to endoglycosidase H (endo H), whereas most but not all of the N-linked oligosaccharides on the 120-kDa monomer were resistant to this enzyme, indicating their processing from high-mannose to complex forms. The endo H sensitivity of the oligomer forms present after a pulse-label and a 3-h chase was assessed. The results demonstrate that, as expected, more N-linked chains were removed by endo H from the 220-kDa oligomer than from the 240-kDa oligomer (Fig. 4).

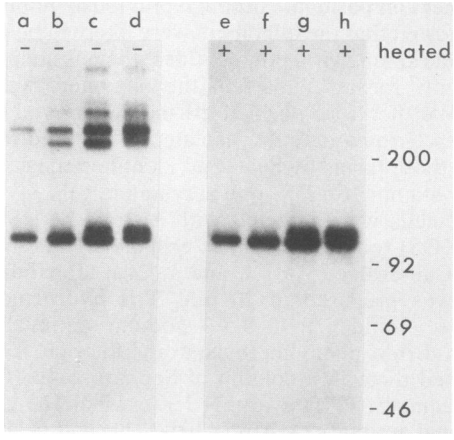

FIG. 2. Kinetics of gB oligomer formation. Immunoprecipitation by using MAb II-105-2 was performed on extracts of HSV-1(KOS)-infected HEp-2 cells labeled at 6 h after infection by a 10-min pulse of [^{35}S]methionine (lanes a and e), followed by a chase in nonradioactive medium for 30 min (lanes b and f), 3 h (lanes c and g), or 18 h (lanes d and h). The protocols for labeling and sample preparation were previously described (6). Samples in lanes a to d were not boiled before SDS-PAGE.

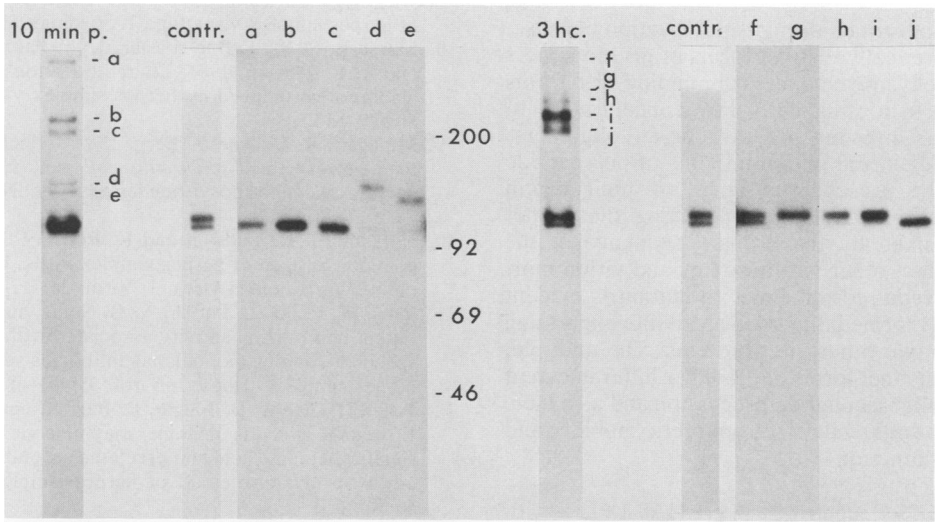

FIG. 3. Forms of gB monomers present in oligomers at different times after gB synthesis. HSV-1(KOS)-infected HEp-2 cells were pulsed for 10 min with [^{35}S]methionine at 6 h after infection (10 min p.) and chased for 3 h (3 h c.). Immunoprecipitations were performed by using MAb II-105-2, and samples were electrophoresed without prior heating. Materials eluted from the gel at positions corresponding to bands a to e in the sample pulsed for 10 min and bands f to j in the sample chased for 3 h were heated and subjected to a second SDS-PAGE (lanes a to e and f to j, respectively). The control lanes (contr.) show gB precipitated from the 3-h chase extract and heated before SDS-PAGE.

The results presented above demonstrate that oligomerization of gB occurs shortly after polypeptide synthesis and before the processing of N-linked oligosaccharides from high-mannose to complex forms. To determine whether N-linked glycosylation was required for oligomer formation, HEp-2 cells infected with HSV-1(KOS) were incubated with tunicamycin from 2 to 6 h after infection. Extracts of the infected cells were then prepared directly for SDS-PAGE and analyzed by Western blotting with an anti-gB antiserum. gB made in the presence of tunicamycin (and of the size expected for gB devoid of N-linked oligosaccharides) formed heat-dissociable oligomers appropriately reduced in apparent size (Fig. 5).

Conclusions. Multiple forms of gB oligomers were detected by SDS-PAGE of unheated samples. The two faster-migrating forms are probably homodimers of gB; this is based on apparent molecular weights and failure to detect other viral or cell proteins in the dissociated purified dimers. It seems likely that the gB dimers exist in the infected cell

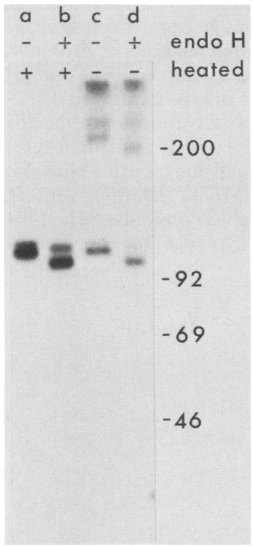

FIG. 4. Sensitivity of gB oligomers and monomers to endo H. Immunoprecipitations were performed by using MAb II-105-2 on an extract from HSV-1(KOS)-infected HEp-2 cells which had been pulsed for 10 min with [^{35}S]methionine at 6 h after infection and then chased for 3 h. Immunoprecipitates were treated with endo H (lanes b and d) or mock incubated (lanes a and c). Samples in lanes c and d were not boiled before SDS-PAGE.

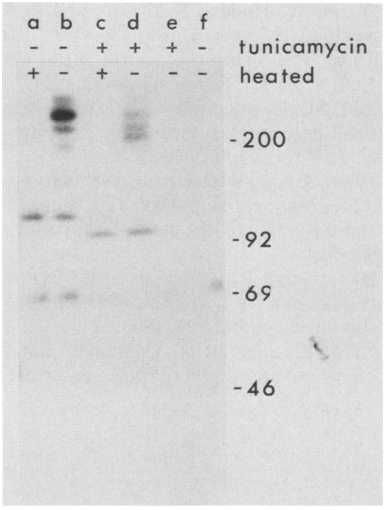

FIG. 5. Effect of tunicamycin on formation of gB oligomers. HEp-2 cells infected with HSV-1(KOS) were treated with tunicamycin (2 μg/ml) from 2 h after infection (lanes c and d) or control incubated (lanes a and b). Lanes e and f show samples obtained from uninfected cells which were either untreated (lane f) or treated with tunicamycin (lane e). Extracts were prepared by solubilizing the cells directly in SDS-PAGE sample buffer at 6 h after infection. Samples were either unheated (lanes b, d, e, and f) or boiled (lanes a and c) before electrophoresis. Proteins were transferred from the gel to nitrocellulose paper by the Western blot procedure and incubated with an anti-gB rabbit antiserum. Antibodies in the antiserum reacted with a cell protein of about 69 kDa as well as with gB.

and virion and are not formed during solubilization because, although electrophoretically distinct forms of gB monomers coexist in infected cells, mixed dimers containing both forms were not detected. In a sufficiently low concentration of SDS, most gB was present in extracts as oligomers. Dimerization of gB occurred within minutes of polypeptide synthesis and in the presence of doses of tunicamycin sufficient to prevent glycosylation, indicating that dimer formation is determined by the primary sequence of the polypeptide. That most other viral proteins and virion morphogenesis are not required for dimer formation is evident from findings that gB formed dimers in a cell line expressing only gB and one or two other viral proteins. The multiple, electrophoretically distinct forms of gB dimer differ in extent or rate of N-linked oligosaccharide processing and also have other differences that influence electrophoretic mobility and may be pertinent to function.

We thank A. Minson for supplying anti-gH MAb LP11 and B. Roizman for plasmids containing the genes for gB and ICP4. The excellent technical assistance of Y. Orandi is gratefully acknowledged. Expert secretarial assistance was provided by J. Hoshizaki.

L.C.W. was a recipient of an EMBO long-term fellowship. This work was supported by Public Health Service grants R01 CA 21776 and P01 CA 19264 from the National Cancer Institute and by a grant from the American Cancer Society.

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