Identification of Polypeptide Precursors to HSV-1 Glycoproteins by Cell-free Translation

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SUMMARY

Using antisera of limited specificity we have detected among the in vitro translation products of HSV-1 RNA, polypeptides antigenically related to infected cell glycoproteins. The results obtained suggest that non-glycosylated polypeptide precursors of mol. wt. 85000 and 52000 correspond to infected cell glycoproteins of mol. wt. 120000 to 126000 and 56000 to 68000 respectively.

The recognition of the important role of glycoproteins in virus infectivity and antigenicity has prompted detailed study of the structure and biosynthesis of these species in many systems, including cells infected by herpes simplex virus (HSV). The mature, fully glycosylated forms of the glycoproteins of HSV-1 so far identified have been designated gA, gB, gC (having apparent mol. wt. in the range 110000 to 130000), gD (approx. 60000) and gE (80000) (Spear, 1976; Baucke & Spear, 1979; Heine et al., 1974).

Two methods have been previously employed to study the precursors to these glycoproteins in infected cells. A number of workers have used drugs, e.g. glucosamine, deoxyglucose (Knowles & Person, 1976; Courtney et al., 1973; Courtney, 1976) and, more recently, tunicamycin (Pizer et al., 1980) which inhibit to varying degrees the addition of carbohydrate residues to polypeptide chains. Under these conditions the appearance of new polypeptide species, coincident with the disappearance of known glycoprotein species, has been observed and these new, lower mol. wt. species have been taken to represent under-glycosylated or unglycosylated forms of the corresponding mature glycoprotein. The second approach has been immunoprecipitation, with glycoprotein-specific antisera, of pulse-labelled infected cell polypeptides (Spear, 1976; Cohen et al., 1978), thus allowing identification of the immediate precursors to the mature glycoproteins. However, these species are presumably partially glycosylated intermediates, as it is likely that carbohydrate addition begins before the polypeptide chain is complete (Rothman & Lodish, 1977). At least some of the species identified in drug-treated cells also contain some carbohydrate: for example a new polypeptide detected by Courtney et al. (1973) after deoxyglucose treatment could still be labelled with radioactive glucosamine. Moreover, as always in drug-treated cells, the possibility of unsuspected perturbations is difficult to exclude. Therefore, neither of the above approaches has allowed unambiguous assignment of molecular weights to the polypeptide moieties of HSV-1 glycoproteins. This information would be useful both for more detailed studies into glycoprotein structure and for mapping studies in order that gene size can be estimated.

We have, therefore, taken a third approach to the examination of HSV-1 glycoprotein precursors. When mRNAs are translated in a cell-free protein synthesizing system, primary translation products can be obtained; glycosylation of polypeptides would not be expected in the absence of microsome supplements (Rothman & Lodish, 1977). Faithful translation of HSV-1 RNA in cell-free systems, including rabbit reticulocytes, has previously been demonstrated (Preston, 1977, 1979; Cremer et al., 1977; Inglis & Newton, 1981) and we report here the use of the rabbit reticulocyte system to identify primary, unmodified translation products related to two of the major HSV-1 glycoproteins.
Fig. 1. HSV-1-infected cell polypeptide patterns in vivo and in vitro. HSV-1-infected cells (10 p.f.u./cell) were labelled 4 to 8 h post-infection with a mixture of $^3$H-labelled amino acids (50 μCi/ml) (track 1, 4 x 10^4 cells, 120000 ct/min), with $[^3]$Hglucosamine (200 μCi/ml) (track 2, 2.5 x 10^5 cells, 12000 ct/min) or with $[^3]$S]methionine (5 μCi/ml) (track 4, 7.5 × 10^3 cells, 20000 ct/min). Cytoplasmic RNA from HSV-1-infected cells 8 h post-infection was translated in the rabbit reticulocyte lysate system containing $[^3]$S]methionine (track 3, 20000 ct/min). Separation was on a 10% polyacrylamide slab gel and labelled polypeptides were detected by fluorography (Laskey & Mills, 1975). Mol. wt. (shown x 10^-3) were estimated from the migration of marker polypeptides of known mol. wt. electrophoresed on the same gel. Polypeptides appearing both in vitro and in vivo (tracks 3, 4) are marked --; those appearing in only one case are marked ▪. Glycoprotein designations were made on the basis of relative mobilities according to the nomenclature adopted by Spear (1976).

Procedures for growth of suspension culture L cells (LS cells) and HSV-1 strain Fra, for RNA preparation, for cell-free translation in the mRNA-dependent rabbit reticulocyte lysate system of Pelham & Jackson (1976) and for polyacrylamide gel analyses have all been described previously (Inglis & Newton, 1981).

HSV-1-infected cell glycoproteins, labelled with $[^3]$Hglucosamine, were detected after polyacrylamide gel separation (Fig. 1, track 2) as three bands with apparent mol. wt. between 120000 and 135000, and a further diffuse band corresponding to the mol. wt. range 60000 to 70000. These glycoprotein assignments are quite compatible with published data from several laboratories (Heine et al., 1974; Spear, 1976; Cohen et al., 1978). The major, high mol. wt. region of glucosamine labelling, containing gA, gB and gC (Fig. 1, track 2), corresponds with an important area of difference between in vivo and in vitro $[^3]$S]methionine labelling patterns.
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(Fig. 1, track 3, 4): co-migrating translation products are distinctly lacking in this region of the gel, most probably, as expected, due to the lack of production of mature glycosylated products in the in vitro system. A number of species were, however, present among the in vitro products but not in the infected cell and it would be anticipated that these should include the unmodified polypeptide moieties of the virus glycoproteins. Similar conclusions have been reached by other investigators (Preston, 1977; Cremer et al., 1977) working with cell-free translation systems, but no evidence has been reported which relates specific in vitro products to particular infected cell glycoproteins.

In order to determine whether any of the in vitro products were related to the major glycoproteins seen in infected cells, antisera to individual virus glycoproteins were employed. These antisera were a generous gift from Professor D. Watson of Leeds University. Anti-Band II antiserum (Watson & Wildy, 1969) had been prepared against a single precipitin band; this antigen is reported to share immunological properties with the type-common structural glycoprotein of 59 000 mol. wt. (and its 52 000 glycoprotein precursor) found in infected cells (Cohen et al., 1978). Anti-VP 7/8 antiserum (Powell et al., 1974) is a type-specific antiserum prepared against the major glycoprotein region of HSV-1 polypeptide profiles corresponding to VP 7/8 (mol. wt. approx. 126 000) in the Spear & Roizman (1972) nomenclature.

Mock-infected and HSV-1-infected cells, labelled with [35S]methionine from 6 to 8 h post-infection were lysed with sodium deoxycholate and Triton X-100 (Inglis & Mahy, 1979) and the lysate centrifuged. Samples of supernatant (soluble antigen) were treated with either anti-Band II antiserum, anti-VP 7/8 antiserum or control rabbit pre-immune serum, followed by adsorption on to Sepharose-linked Staphylococcal protein A. Samples of reticulocyte translation mixes were similarly treated after 60 min incubation in the presence of either mock-infected or HSV-1-infected cell RNA. The precipitated antigens were eluted from the adsorbent (Inglis & Mahy, 1979) and subjected to polyacrylamide gel electrophoresis (Fig. 2). Tracks 6 and 7 show that anti-Band II and anti-VP 7/8 antisera indeed react with polypeptides labelled in whole cells which co-migrate with known glycoproteins. Thus, anti-Band II antiserum specifically precipitated polypeptide species in two broad bands, migrating with apparent mol. wt. of approx. 56 000 to 58 000 and 62 000 to 68 000 (presumably representing different stages of glycosylation of gD), while anti-VP 7/8 antiserum precipitated two major bands which co-migrate with the major high mol. wt. virus glycoproteins, having apparent mol. wt. between 120 000 and 126 000 and which we believe to be gA and gB. Anti-VP 7/8 also reacted to a lesser extent with a polypeptide migrating with the major capsid polypeptide (VP 5) at mol. wt. 155 000, indicating that this serum may have contained some antibodies directed against VP 5. As VP 7/8 antigen for inoculation of the rabbit had been prepared by excision of a gel peak adjacent to, and not wholly resolved from, the peak containing major capsid antigen (Powell et al., 1974) this seems quite possible.

Fig. 2, tracks 14 and 15 show the equivalent precipitations of in vitro translation products of infected cell RNA. Above the background common to both tracks, one specifically precipitated band can be identified in each track, in each case migrating with a lower apparent mol. wt. than the similarly reactive in vivo counterpart (Fig. 2, track 6, 7). Anti-Band II antiserum (Fig. 2, track 14) specifically precipitated a polypeptide from the translation products with an apparent mol. wt. of approx. 52 000 and anti-VP 7/8 specifically reacted with a species of around 85 000 mol. wt. (Fig. 2, track 15). No precipitation of in vitro synthesized VP 5 by anti-VP 7/8 was detected but this would not be unexpected as anti-VP 5 activity was a minor component of the antiserum (Fig. 2, track 7) and VP 5, being a high mol. wt. polypeptide, is under-represented in cell-free translation products (Fig. 1, track 3). The in vitro synthesized polypeptide species immunoprecipitated by these antisera, therefore, share antigenic determinants with HSV-1 glycoproteins and are most likely to represent the primary, unmodified precursors to these.
Fig. 2. Glycoprotein identification by precipitation with antisera of limited specificity. Mock-infected LS cells (tracks 1 to 4) and HSV-1-infected LS cells (tracks 5 to 8) were labelled with $[^{35}\text{S}]$methionine 6 to 8 h post-infection and harvested. An aliquot was prepared for polyacrylamide gel electrophoresis (track 1, 5) and the remainder lysed for soluble antigen preparation. Equal amounts of soluble antigen were treated with either anti-Band II antiserum (track 2, 6), anti-VP 7/8 antiserum (track 3, 7) or control pre-immune serum (track 4, 8) and the immune complexes collected by addition of Sepharose-linked Staphylococcal protein A. Antigens were eluted in equal volumes of sample buffer and aliquots were applied to the gel. For tracks 9 to 16 cell-free translation reactions were carried out in the presence of $[^{35}\text{S}]$methionine, directed by cytoplasmic RNA from mock-infected (tracks 9 to 12) and 8 h HSV-1-infected cells (tracks 13 to 16). A portion of each incubation mix was electrophoresed directly (track 9, 13); aliquots of the remainder were treated with the antisera as above (anti-Band II (track 10, 14), anti-VP 7/8 (track 11, 15) and pre-immune (track 12, 16)) and the respective antigens collected and eluted as before. Equal amounts of each were applied to the gel. Separation was on a 15% gel and $[^{35}\text{S}]$methionine-labelled polypeptides were detected by autoradiography.

The 52 000 mol. wt. species, the putative gD precursor, is apparent in gel tracks showing total translation products (Fig. 1, track 3 and Fig. 2, track 13). However, the 85 000 mol. wt. species is not a major translation product, although in infected cells the 120 000 and 126 000 mol. wt. glycoproteins are major $[^{35}\text{S}]$methionine-labelled species (Fig. 1, track 4). Two
possible reasons why the putative precursor is a minor translation product are, firstly, that glycoprotein mRNAs, being membrane-associated during translation in whole cells, may be poorly recovered from cell extracts, and, secondly, that this again is a relatively large polypeptide and consequently may be under-represented among in vitro products either as a result of RNA degradation or preferential translation of shorter mRNAs.

The data presented, however, are consistent with there being non-glycosylated polypeptide precursors of mol. wt. approx. 85 000 and 52 000 corresponding to infected cell glycoproteins of respectively 120 000 to 126 000 (gA and gB) and 56 000 to 68 000 (gD) mol. wt. as judged by migration in SDS gels. The finding that anti-VP 7/8 antisemur precipitated both gA and gB from infected cells but only a single detectable polypeptide from in vitro translation products supports the notion that mature glycoproteins gA and gB represent different degrees of glycosylation of the same polypeptide chain (Eberle & Courtney, 1980). The primary translation products detected are similar in size to the 50 000 and 85 000 mol. wt. polypeptides induced in HSV-1-infected cells by tunicamycin (Pizer et al., 1980). The larger species also corresponds well with that induced by deoxyglucose treatment in spite of the fact that this latter species still contains some carbohydrate; this species was originally estimated to be 85 000 (Courtney et al., 1973) and subsequently 92 000 mol. wt. (Courtney, 1976). However, the polypeptides synthesized in vitro, which should reflect directly the size of the genes encoding them, may well be larger than any unglycosylated species detectable in vivo. These membrane glycoproteins, in common with several other glycoproteins, quite probably contain an N-terminal ‘signal sequence’ (usually around 20 amino acids long) which seems to be required to initiate the membrane insertion process (Blobel & Dobberstein, 1975). This sequence may subsequently be cleaved off in vivo, prior to completion of synthesis of the polypeptide chain (Lingappa et al., 1978) even in the absence of glycosylation (Garoff & Schwarz, 1978). Primary translation products made in vitro on the other hand would be expected to retain such a sequence.

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