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SYNTHESIS OF GLYCOPROTEINS OF SYNCYTIAL AND NON-SYNCYTIAL STRAINS OF HERPES SIMPLEX VIRUS TYPE 1 AND THEIR TRANSPORT TO THE PLSAMA MEMBRANE

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Summary. — Underglycosylated form of glycoprotein C (gC) synthesized in the presence of tunicamycin was found on the surface membrane of BHK cells infected with the non-syncytial (non-syn) strain Kupka of herpes simplex virus type 1 (HSV-1). Such form of gC was not detected in the plasma membrane of tunicamycin-treated cells infected with the syncytial (syn) HSZP strain of HSV-1. In comparison to the non-syn strain Kupka, a smaller amount of glycoproteins of the syn HSZP strain was detected on the surface membrane of Vero cells. The same results were obtained using BHK cells, in which no polykaryocytes were induced. The individual glycoproteins of both strains under study had been synthesized and accumulated in a similar way in infected cells. However, at least in Vero cells a delay in the processing of syn strain HSZP gylocoproteins was observed.

Key wods: herpes simplex virus type 1; glycoproteins; transport to plasma membrane

Introduction

In the course of HSV-1 infection of permissive cells at least 4 viral glycoproteins are synthesized designated as gB, gC, gD and gE (Spear, 1976; Baucke and Spear, 1979; Eberle and Courtney, 1980). These glycoproteins became components not only of the viral envelope but also of the cell membranes (Spear *et al.*, 1970; Spear and Roizman, 1972; Norrild *et al.*, 1978; Compton and Courtney, 1984).

The development of syncytial type of cytopathic effect requires mature viral glycoproteins including their transport and insertion to the cell surface membrane (Keller, 1976; Kousoulas *et al.*, 1978; 1983*a*; Serafini-Cessi *et al.*, 1983).

The analysis of recombinants between a mutant not inducing gC synthesis and a mutant lacking mature form of gB has shown that gB is probably responsible for fusion initiation, while gC suppresses syncitium formation induced in some cell lines by certain HSV-1 laboratory-passaged strains (Manservigi *et al.*, 1977). The gB is also essential for early stages of infection —

— it is responsible for fusion of virion envelope with the host cell surface membrane (Sarmiento *et al.*, 1979; Little *et al.*, 1981). However, Honess *et al.* (1980) have shown that some recombinant clones of HSV-1 not able to synthesize gC do not induce polykaryocytes in BHK cells. The original notion was further weakened, when monoclonal antibodies to gD had been found to inhibit fusion in contrast to anti-gB monoclonal antibodies which did not exhibit such ability (Noble *et al.*, 1983).

The glycoproteins of syn strain of HSV-1 are present on the surface membrane at the same interval post-infection (p. i.) in lower amounts as glycoproteins of the parent non-syn strains. The difference in their transportation efficiency may be due to decreased rate of processing of HSV-1 syn strain glycoproteins (Person *et al.*, 1982).

In our work we compared the transport of viral glycoproteins to the plasma membranes of BHK and Vero cells infected with the syn strain HSZP or with the non-syn strain Kupka.

Materials and Methods

Cells. African green monkey kidney (Vero) and baby hamster kidney (BHK) cells were grown to confluency in Petri dishes (5 cm in diameter) in Eagle's basal medium (BEM) supplemented with 10% inactivated bovine serum (IBS).

Viruses and infection. HSV-1, strain Kupka (isolated by Dr. R. Benda, Prague) and HSV-1 strain HSZP (Szántó et al., 1972) were used throughout. Strain HSZP induced formation of large polykaryocytes in Vero cells, in contrast to cell rounding in BHK cells. Strain Kupka induced polykaryocytes neither in Vero, nor in BHK cells. The confluent cell monolayer was infected with the virus dose of 5 PFU/cell. After 60 min adsorption at 37 °C, the inoculum was removed and replaced with maintenance medium (BEM supplemented with 5% IBS).

Metabolic and surface labelling of cells. At different intervals p.i., maintenance medium was removed and cell cultures were rinsed with prewarmed phosphate-buffered saline (PBS). For long-term labelling 2 ml of methionine-free medium supplemented with 1% IBS and containing 0.2 MBq/ml of ^{35}S-methionine (specific activities 29.1 GBq/mmol) was added for indicated intervals. For pulse labelling 0.5 MBq of ^{35}S-methionine was used for 2hr. For pulse-chase protocol, monolayers were rinsed three times with prewarmed maintenace medium containing 150 μg/ml of cycloheximide (Fluka) and incubation was continuted in the presence of cycloheximide for indicated chase intervals. At the end of the long-term pulse or pulse-chase labelling experiments, the monolayers were rinsed three times with cold PBS, scrapped off and pelleted.

Radioiodination of cell surface proteins was done by iodogen method in a slight modification (Hudson and Hay, 1980). Briefly, the cultures of non-infected BHK and Vero cells or infected BHK cells at various intervals p.i. were rinsed with Ca^{2+} and Mg^{2+} -free PBS and detached with versene (5 mmol/l EDTA in Ca^{2+} and Mg^{2+} -free PBS). Approximately $3-5 \times 10^6$ of cells in 1 ml of PBS were added to the glass Petri dish (3 cm in diameter) containing 300 μg of Iodogen (Pierce, Rockford) and 9 MBq of $Na^{125}I$ (specific activities 3.15 GBq/ml). The reaction continued for 20 min at room temperature with continual swirling. Then, the labelled cells were washed three times with PBS and subjected to radioimmunoprecipitation assay. In order to prevent the formation of large fragile syncytia and to enable the comparison of Vero cells infected with non syn or syn strain of HSV-1, infected cells at 1 hr p.i. were detached with versene, diluted in maintenance medium and kept in suspension by moderate shaking. At indicated times p.i., the cells were sedimented and washed three times with PBS and subjected to radioiodination as described above.

Radioimmunoprecipitation assay and SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Details of radioimmunoprecipitation assay were described earlier (Raučina *et al.*, 1984). Previously characterized anti-gC/gB and anti-envelope (anti-env) sera (Leššo *et al.*, 1976; Raučina *et al.*, 1984) were used. Samples were analysed by SDS-PAGE in 8% polyacrylamide gel (Matis and Rajčáni, 1980). Dried gels were exposed to X-ray Medix rapid films (Hradec Králové) for

4—5 days. Fluorographic enhancement for ^{35}S-labelled samples was provided by 1 mol/l sodium salicylate and for ^{125}I-labelled samples Perlux Extra-rapid X-ray intentification screens were used.

Results

Transport of viral glycoproteins to the plasma membrane in the absence and presence of tunicamycin

The transport of gB and gC of the syn and non-syn strains of HSV-1 synthesized in the absence and presence of tunicamycin to surface membrane of BHK cells was compared (Fig. 1). The precipitation of ^{125}I-labelled surface proteins of cells infected with syn HSZP and non-syn strain Kupka with the anti gC/gB serum has shown that both glycoproteins are present at the cell surface at 20 hr p.i. However, the amount of gC and gB found on the surface of HSZP strain-infected cells was lower than of gC and gB on the plasma membrane of Kupka strain-infected cells. Only a small amount of ^{125}I-labelled protein of apparent M_r of 100 000 synthesized in the presence of tunicamycin was precipitated from the Kupka strain-infected cells with anti-gC/gB serum. Under the same conditions no virus proteins reacting with anti-gC/gB serum were detected in the plasma membrane of HSZP strain-infected cells.

Accumulation of HSV-1 glycoproteins in BHK and Vero cells

The aim of these experiments was to determine the amount of glycoproteins accumulating in BHK and Vero cells at 5, 10 and 15 hr p.i. Cells infected with strain Kupka or HSZP were labelled from 3 hr p.i. with ^{35}S-methionine. At given intervals the long-term labelling was interrupted, the solubilized proteins were precipitated with anti-env serum and analysed in SDS-PAGE. Till 5 hr p.i. only small amounts of gB were synthesized in BHK cells (Fig. 2). Until 10 hr p.i. cells have accumulated gC and gD in addition to gB, but the highest amounts of individual glycoproteins were detected in infected cells by 15 hr p.i.

Similar results were achieved by comparing the accumulation of individual glycoproteins in Vero cells (Fig. 3). At 5 hr p.i. gB and pgB were detected. Both exhibited a higher electrophoretic mobility as compared to those synthesized in BHK cells, which was due to a partial proteolysis (Pereira *et al.*, 1981, 1982). As to 10 hr p.i. also gC and gD were detected, and at 15 hr p.i. the higest amount of individual glycoproteins was observed.

More pronounced differences in the accumulation of gC between both strains under study were found in Vero cells only. As to other gylocoproteins, no such differences in accumulation either in Vero or in BHK cells were detected.

Kinetics of synthesis and stability of viral glycoproteins in BHK and Vero cells

Infected cells were pulse-labelled with ^{35}S-methionine at intervals from 3 to 5, from 8 to 10 and from 13 to 15 hr p.i., respectively. After the pulse,

cells were either processed for radioimmunoprecipitation assay or the glycoproteins labelled between 3—5 or 8—10 hr p.i. were chased up to 15 hr p.i.

A significant amount of gB and gC synthesized in BHK cells from 3 to 5 hr p.i. (Fig. 4). At intervals of 8—10 hr p.i. and of 13—15 hr p.i., all three glycoproteins — gB, gC and gD — were formed. The glycoproteins produced from 3 to 5 and from 8 to 10 hr p.i. were still present at 15 hr p.i., although a decrease in amount of gB had been observed after both chase intervals. The processing of gD was remarkable, at 15 hr p.i. the mature gD prevailed. In addition, a protein of electrophoretic mobility which might correspond to the gB degradation product — gB' was detected after the chase interval (Pereira *et al.*, 1981, 1982; Respess *et al.*, 1984). No essential differences between the strains under study were found in the synthesis and stability of viral glycoproteins in BHK cells.

The results of a similar experiment performed in Vero cells were partially different (Fig. 5). Only a small amount of pgB was detected in Vero cells infected with strain HSZP or strain Kupka by 5 hr p.i. At intervals 8—10 and 13—15 hr p.i., the gB, gC and gD were synthesized at a high rate. Further we compared the amount of individual glycoproteins synthesized during the pulse intervals with that of glycoproteins present in Vero cells after the chase till 15 hr p.i. No gB synthesized between 3—5 hr p.i. was detected at 15 hr p.i. In addition, the amout of gB synthesized from 8 to 10 hr p.i. was reduced, what could be caused by degradation of gB accompanied with appearance of the gB' as a degradation product. The processing rate of gB was also different; while the mature form of gB was present already after the pulse between 8—10 hr p.i. in Kupka strain-infected cells, in HSZP strain-infected cells gB appeared within the subsequent chase interval. The processing of gD in Vero cells had a similar course than in BHK cells: during the chase pgD had undergone posttranslational modification to the mature gD.

The possibility has been tested whether the decreased amount of gB observed at the end of the chase period was due to its secretion into medium. Immunoprecipitation with anti-env serum as well as the analysis of all ^{35}S-labelled proteins present in the medium has not revealed any significant secretion of viral proteins from infected BHK or Vero cells (data not shown).

Transportation kinetics of viral glycoproteins to the plasma membrane of Vero and BHK cells

The glycoproteins in the plasma membrane were detected by immunoprecipitation of ^{125}I-labelled surface proteins from Vero- and BHK-infcted cells. At 5 hr p.i. only gC and gB were found in the plasma membrane of Vero cells (Fig. 6). By 10 hr p.i., the amount of gC and gB had increased and gD also appeared. At 15 hr p.i. the largest amounts of major glycoproteins were detected. The comparison of glycoproteins of strain Kupka with those of strain HSZP at different intervals p.i. confirmed the transportation delay of the latter (mainly that of gB) into the plasma membranes of Vero cells.

Similar results were found following transportation kinetics of viral glycoproteins to the plasma membrane of BHK cells (Fig. 7). At 5 hr p.i. gC and

gB were present on the surface of cells infected with both HSV-1 strains. Their amount has increased until 15 hr p.i. Glycoprotein D was detected on the plasma membrane 10 hr p.i. and later. The delay in transport of HSZP strain glycoproteins to the plasma membrane in comparison with the non-syn Kupka strain glycoproteins was apparent also in BHK cells.

On the surface of infected cells predominantly mature forms of glycoproteins were detected. Only the plasma membrane of HSZP-infected Vero cells contained at 15 hr p.i. a ^{125}I-labelled protein which electrophoretic mobility could correspond to that of pgD.

Discussion

Glycoprotein C contains N-linked as well as O-linked oligosaccharides (Olofson *et al.*, 1981). In the presence of tunicamycin which inhibits the synthesis of N-glycosylated oligosaccharides (Heifetz *et al.*, 1979), an underglycosylated form of gC (M_r = 92 000) is synthesized containing only oligosaccharides bound via O-glycosidic bonds (Wenske and Courtney, 1983). In the presence of tunicamicyn only little amounts of a protein of apparent M_r of 100 000 representing the gC core protein with O-linked oligosaccharides (Fig. 1) has been precipitated with anti-gC/gB serum from the plasma membranes of non-syn strain Kupka-infected BHK cells. The presence of underglycosylated gC on the surface of infected cells indicates that the addition of O-linked oligosaccharides to the core protein may be sufficient for its at least partial transport to plasma membrane. Nevertheless, other non-glycosylated core proteins have not been detected in the plasma membranes (Fig. 1; Norrild and Pedersen, 1982; Norrild *et al.*, 1983). No proteins synthesized in the presence of tunicamycin were precipateted with the anti-gC /gB serum from the surface membranes of BHK cells infected with the syn strain HSZP. Alternatively, Peake *et al.* (1982) and Kousoulas *et al.* (1983*b*) did not find viral glycoproteins in the plasma membranes of cells infected with various HSV-1 strains in the presence of tunicamycin. The absence of the underglycosylated gC on the surface membrane may be caused by the lack or decreased amount of O-linked oligosaccharides in the gC of some HSV-1 strains (Kumarasamy and Blough, 1982; Raučina *et al.*, 1984). This can be part due to the impaired transport of mature gC of syn strains to the plasma membrane (Person *et al.*, 1982; Fig. 7).

The observed differences between the syn and non-syn HSV-1 strains in the transport eficiency of gB and gC to surface membrane promoted us to a more detailed analysis of the kinetics of glycoprotein transport to the surface of infected cells. The glycoproteins of both strains under study were inserted into the plasma membrane of BHK and Vero cells in accordance with previously published kinetic data (Norrild *et al.*, 1987). The gB and gC were detected already at 5 hr p.i. (Figs 6 and 7). It can be assumed that at least a part of these glycoproteins comes from the virus inoculum (Para *et al.*, 1980; Peake *et al.*, 1982). At 10 hr p.i. the amount of gB and gC has considerably increased and gD apeared. At 15 hr p.i. the amount of viral glycoproteins was the highest. In both cell lines infected with the syn strain HSZP a delay was

observed in glycoprotein transport to surface membranes as compared with that in Kupka strain-infected cells. This finding is in accordance with the results of Person *et al.* (1982). The decreased insertion of the syn HSZP strain glycoproteins into the plasma membranes of BHK cells, in which the polykaryocytes were not induced, confirmed that the impaired transport of syn strain glycoproteins was their general property not always accompanied by formation of syncytia. The difference in accumulation of glycoproteins in plasma membrane between the syn and non-syn strains was not due to release of glycoproteins from the cells. After a chase interval up to 15 hr p.i., no significant amounts of individual viral glycoproteins were detected in the medium indicating their predominant cell-association.

Similarly as Person *et al.* (1982) we also observed a delayed processing of certain glycoproteins at least in Vero cells infected with the HSZP strain. The rate of glycoprotein processing can considerably influence the transport to the plasma membrane because the glycoproteins detected at the cell surface were predominantly mature ones (Figs 6 and 7; Person *et al.*, 1982). The difference between the syn and non-syn strains in the processing and transport to the plasma membrane of gC and gD was not as clear-cut as observed with gB. The degree of glycoprotein separation achieved in our gels did not allow to differentiate the glycoprotein precursors synthesized in BHK cells and to analyse their processing in more detail.

The glycoprotein pool increased by time of infection, but the differences in the amounts of mature glycoproteins found between the syn and non-syn strains were not very apparent despite of differences in their processing rate (Figs 2 and 3). The discrepancy between the pool size of mature glycoproteins (mainly gB) of the syn strain HSZP and their transportation rate to the surface membrane could mean that processing and membrane insertion of HSV-1 glycoproteins are rather independent. The mature glycoproteins are probably not always transported to the plasma membranes by the same efficiency and, on the other hand, under certain circumstances underglycosylated glycoproteins may also appear on the surface of infected cells (Glorioso *et al.*, 1983).

Our results support the notion that polykaryocyte formation is a complex event governed by the quality and quantity of viral glycoproteins as well as by the components of host cell plasma membranes (Lee and Spear, 1980).

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Description of Figures (Plates XXVIII–XXXIV):

Fig. 1. Transport of gC and gB to plasma membrane of BHK cells in the absence and presence of tunicamycin. Cells infected either with the syn strain HSZP (H) or with the non-syn strain Kupka (K) were ^{125}I-labelled at 20 hr p.i. The proteins were precipitated with an anti-C/gB serum and analysed in SDS-PAGE. Infected cells were incubated in the absence (−TM) or since 4 hr p.i. in the presence (+TM) of tunicamycin (4 μg/ml). The position of gB, gC and of the protein M_r 100 000 is indicated on the right.

Fig. 2. Accumulation of HSV-1 glycoproteins in BHK cells. Infected cells were labelled with ^{35}S-methionine from 3 to 5 hr p.i. (5), from 3 to 10 hr p.i. (10), and from 3 to 15 hr p.i. (15). Solubilized proteins were precipitated with anti-env serum. N — non-infected BHK cells.

Fig. 3. Accumulation of HSV-1 glycoproteins in Vero cells. For explanation see Fig. 2.

Fig. 4. Synthesis and stability of HSV-1 glycoproteins in BHK cells. Infected cells were pulse-labelled with ^{35}S-methionine between 3–5 hr p.i. (3–5), 8–10 hr p.i. (8–10) or 13–15 hr p.i. (13–15). The cells were either immediately after the pulse-labelling (P) processed for radioimmunoprecipitation assay as described in Materials and Methods and precipitated with anti-env serum, or they were further chased (CH) in radiolabel-free medium until 15 hr p.i. The position of mature gB, gC and gD, precursors pgB and pgD and of degradation product gB' is indicated on the right.

Fig. 5. Synthesis and stability of HSV-1 glycoproteins in Vero cells. The gB-related proteins are marked (■). For further explanation see Fig. 4.

Fig. 6. Transport of HSV-1 glycoproteins to surface membrane of Vero cells.
Cells were infected with strain Kupka or with the strain HSZP and ^{125}I-surface labelled at 5 hr (5), 10 hr (10) or 15 hr p.i. (15). Solubilized proteins were precipitated with the anti-env serum.

Fig. 7. Transport of HSV-1 glycoproteins to sufrace membrane of BHK cells.
For explanation seee Fig. 6.