Comparison of heparin-sensitive attachment of pseudorabies virus (PRV) and herpes simplex virus type 1 and identification of heparin-binding PRV glycoproteins

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To determine whether heparan sulphate residues on the cellular surface could serve as an attachment receptor for pseudorabies virus (PRV), the effect of heparin on PRV in plaque reduction and adsorption tests was investigated. PRV was significantly less sensitive to heparin than herpes simplex virus type 1 (HSV-1). At concentrations of 500 µg/ml heparin the number of plaques formed by PRV was reduced to 7% of the untreated control whereas the number of plaques formed by HSV-1 was reduced to below 0.1%. Adsorption of PRV to host cells was also less sensitive to heparin treatment than was adsorption of HSV-1.

Experiments concerning the binding sites of PRV showed that heparin binds to the disulphide-linked glycoprotein complex gII (PRV gB), gIII (PRV gC) and probably gV.

The first events in the life cycle of a herpesvirus are attachment to the cellular surface and entry of the virus into the cytoplasm of its host cell. Many studies on the entry of herpesviruses into the cell have been done (Epstein et al., 1964; Morgan et al., 1968; Dales & Silverberg, 1969; Smith & deHarven, 1974; DeLuca et al., 1981; Rosenthal et al., 1989), but little was known about attachment (Fuller & Spear, 1985; Johnson & Ligas, 1988) until it was shown recently by WuDunn & Spear (1989) that heparan sulphate proteoglycans present on the surface of many cell types (Höök et al., 1984) serve as an attachment receptor for herpes simplex virus (HSV) types 1 and 2. They used heparin, a glycosaminoglycan structurally related to heparan sulphate, to block specifically the attachment of HSV to the cell surface and to reduce the number of plaques formed by HSV on HEp-2 cells.

To determine whether heparan sulphate can also serve as an attachment receptor for pseudorabies virus (PRV), a herpesvirus of swine causing Aujeszky's disease in pigs, the effect of heparin on plaque formation and adsorption of PRV (strain Ka), was evaluated and compared with HSV-1 strain HFEM.

Fig. 1 shows that heparin inhibited plaque formation (a) and virus adsorption (b) of PRV to RK13 cells, a rabbit kidney cell line. Interestingly, both viruses were affected by heparin treatment, but PRV was significantly less sensitive to heparin than was HSV-1. The number of plaques formed by PRV was reduced to 10% of the untreated control (100%) by 10 µg/ml heparin, but could not be reduced below 7% even with concentrations up to 500 µg/ml, whereas the number of plaques formed by HSV-1 on HEp-2 cells was reduced to approximately 1% of the untreated control value by 10 µg/ml heparin and was reduced even further to below 0.1% with concentrations of 500 µg/ml. Adsorption of HSV-1 to HEp-2 cells was reduced to 3% of the untreated control (100%) by a concentration of up to 500 µg/ml heparin, whereas adsorption of PRV to RK13 cells was reduced to 45% of the untreated control. Therefore it can be concluded that either the virus attachment structures of PRV are less sensitive to heparin or that adsorption of PRV can occur in more than one way, as reported by Schreurs et al. (1988). In the latter case, one method must be insensitive to heparin.

Since viral glycoproteins play a major role in virus attachment and entry (Sarmiento et al., 1979; Little et al., 1980; Fuller & Spear, 1985; Desai et al., 1988; Johnson & Ligas, 1988; Schreurs et al., 1988; Johnson & Spear, 1989) we determined which of the PRV glycoproteins have an affinity for heparin. In our experiments, [3H]glucosamine-labelled PRV proteins were bound to heparin-acrylic-beads (HAB) and subsequently analysed in SDS–PAGE and two-dimensional (2D) PAGE.

Fig. 2 shows PRV proteins bound by HAB separated on a 10% SDS–PAGE gel. Under non-reducing conditions, three [3H]glucosamine-labelled proteins with Mr values of approximately 150K, 75K and 62K can be detected (lane 1). In a control experiment, addition of heparin (1 mg/ml) to the binding assay inhibited virtually...
Fig. 1. Effect of heparin on plaque formation and adsorption of PRV and HSV-1. (a) Effect of heparin on plaque formation. Virus from a stock solution was diluted in logarithmic steps in Eagle's MEM containing the appropriate concentration of heparin. After a 30 min incubation at 37 °C, RK13 or HEp-2 cells were inoculated with 0.5 ml of the virus solution and incubated for 30 min at 37 °C. The inoculum was removed and the cells were rinsed twice with 1 ml of Eagle's MEM containing the appropriate concentration of heparin. Subsequently the cells were overlaid with Eagle's MEM containing 10% foetal calf serum and 0.7% (w/v) agarose. Plaques were counted after 3 or 4 days. Incubation of PRV (○) and HSV (●) with heparin during infection resulted in a reduction of the number of plaques formed by both viruses. Incubation of PRV-infected cells with heparin after a 30 min adsorption interval (∇) showed no effect, indicating that heparin acts in an event early in infection, e.g. attachment or penetration. (b) Effect of heparin on virus attachment. Experiments were done as described by WuDunn & Spear (1989) with minor modifications. In brief, RK13 or HEp-2 cells were pretreated for 15 min at 37 °C with Eagle's MEM containing 1% foetal calf serum and bovine serum albumin (5 mg/ml). Afterwards the cells were inoculated for 30 min at 37 °C with [3S]methionine-labelled virus, which had been preincubated for 30 min at 37 °C in Eagle's MEM containing the appropriate concentration of heparin. The inoculum was removed and the cells were washed four times with phosphate-buffered saline (PBS). Cells were lysed in PBS containing 1% SDS and 1% Triton X-100. The lysates were dissolved in INSTA-GEL (Packard Instrument Company) and radioactivity was measured in a Packard scintillation counter. Treatment with heparin inhibited virus adsorption of both PRV (○) and HSV (●). Adsorption of PRV to cells is significantly less sensitive to heparin treatment than is adsorption of HSV-1, a pattern similar to the results for plaque formation.

Fig. 2. Separation of [3H]glucosamine-labelled PRV proteins by SDS-PAGE. After equilibrating 100 mg of HAB (Sigma) with PBS, 100 µl PBS or PBS containing heparin (2 mg/ml) and 100 µl of [3H]glucosamine-labelled PRV were added and incubated for 120 min on ice. Thereafter the beads were washed seven times with 500 µl NT buffer (50 mM-Tris- HCl pH 7·4, 150 mM-NaCl). After sedimenting the beads and discarding the washing buffer, bound proteins were released from the beads with incubation at 8 M-urea containing 2% SDS for 5 min at 100 °C. Beads were sedimented by centrifugation for 5 min at 15000 g and the supernatant was analysed on a 10% SDS-PAGE gel. Samples in lanes 4 and 5 were reduced by treatment with 0.025 M-DTE. Samples in lanes 1, 2 and 3 are non-reduced. Lanes 1 and 4 (HAB) show that under non-reducing conditions proteins with Mr values of approximately 150K, 75K and 62K were precipitated with HAB. Under reducing conditions the 150K band is smaller and two new proteins with Mr values of 98K and 56K are detectable. Lane 2 (HAB + heparin) shows that no protein bound to HAB when heparin was added to the binding assay at 1 mg/ml. In lanes 3 and 5 the proteins of the whole Triton-solubilized envelope fraction are demonstrated for comparison. Markers to the left and to the immediate right indicate PRV proteins. Markers to the extreme right are reference Mr markers.
Fig. 3. Two-dimensional PAGE of [3H]glucosamine-labelled PRV proteins precipitated by HAB. [3H]Glucosamine-labelled PRV proteins were precipitated with HAB as described in Fig. 2 but released from the beads by incubation with lysis buffer (9.5 M-urea, 2% Triton X-100, 1.6% Ampholines pH 5 to 8, 0.4% Ampholines pH 3 to 10, 5% 2-mercaptoethanol) for 30 min at 37 °C. Two-dimensional PAGE was performed as described by O'Farrell (1975) with minor modifications. Gels containing a mixture of 1.6% carrier Ampholines pH 5 to 8 and 0.4% pH 3 to 10 (Pharmacia) were used. Samples were focused for 18 h at 400 V and 1 h at 800 V, yielding 8000 Vh. Focused gels were stored at -70 °C until the second dimension separation was done, on 10% SDS-polyacrylamide gels. Isoelectric focusing (IEF) gels were equilibrated for SDS-PAGE by incubation in SDS equilibration buffer (O'Farrell, 1975) prior to use. The pH gradient in the focused IEF gel was determined with a Beckman pH meter after slicing the gels into 5 mm sections and incubating them in 8 M-urea for 30 min. The gradient usually spanned 2 pH units and ran between pH 6.0 and 8.0. The peptides with M, values of 123K, 69K and the double row of spots with M, 56K and 58K were identified as glIa, glIb and glIc. With an M, of 75K to 100K gIII is visible on the right side of the gel. The remaining two spots with M, values of about 105K and 56K to 71K were identified as glIV and gV, respectively. The pH measured in the IEF gel is shown across the top of the gel. M, markers are shown on the right.

Fig. 4. Two-dimensional PAGE of immunoprecipitated gIII. [3H]Glucosamine-labelled gIII was immunoprecipitated with a polyclonal goat serum against a denatured E. coli-produced cro-gIIII fusion protein (Ryan et al., 1987) and subsequently analysed in 2D PAGE. Immunoprecipitation was done by the method of Kessler (1975) with the modifications described previously (Hampl et al., 1984), but Protein A-Sepharose CL-4B (Sigma) was used instead of preparations of Staphylococcus aureus. Two-dimensional PAGE was as described for Fig. 3. Two spots with M, values of 97K and 78K to 97K, are visible. By comparing their position on the gel with results published previously (Hampl et al., 1984), they can be identified as gII and gIV, respectively. The pH measured in the IEF gel is shown across the top of the gel. M, markers are shown on the right.

Lukács et al., 1985), the PRV homologue of the glycoprotein B (gB) of HSV (Robbins et al., 1987). Although their M, values would be slightly smaller than those reported previously (Hampl et al., 1984), the 98K band would represent the gIIa peptide and the 56K peptide would be gIIc. gIIb, with an M, estimated to be about 74K would be represented by the band in the 62K range.

To identify the 150K DTE-sensitive protein as well as the 75K band, PRV proteins bound to HAB were separated by 2D PAGE and identified by comparing their position on the gels with the 2D PAGE analysis of PRV glycoproteins (published by Hampl et al., 1984) as well as gels of immunoprecipitated PRV proteins. The three peptides visible in Fig. 3 with M, values of about 123K, 69K and the double row of spots at 56K and 58K could be identified as gIIa, gIIb and gIIc. This proved that the 150K band represents the gII complex. Since 2D PAGE was done under reducing conditions, no complex was visible.

Little is known about the biological functions of PRV gII. As gII shows a significant homology on the amino acid level to HSV-1 gB (Robbins et al., 1987), it can be concluded that it could serve similar functions. HSV-1
gB is highly conserved among herpesviruses and gB homologues have also been described for HSV-2 (Person et al., 1985), human cytomegalovirus (Cranage et al., 1986), varicella-zoster virus (Emini et al., 1987), bovine herpesvirus type 1 (Misra et al., 1988), Epstein–Barr virus (Pellett et al., 1985a), Marek's disease virus (Ross et al., 1989) and equine herpesvirus types 1 and 2 (Meredith et al., 1989; Whalley et al., 1989). HSV gB has been shown to be involved in virus entry into host cells (Little et al., 1980; Pellett et al., 1985b; Cai et al., 1988; Highlander et al., 1988) and virus-induced cell fusion (Cai et al., 1988). Since gB-deficient virions were shown to bind to the cellular surface (Sarmiento et al., 1979; Little et al., 1980; Cai et al., 1988; Highlander et al., 1988), it can be concluded that HSV gB is essential for infectivity at the level of penetration but is not involved in virus attachment to the cell.

As no data are available, concerning the biological functions of PRV gII, no comments about the biological significance of the heparin-binding properties of gII can be made.

The 75K protein could be identified as gIII, which is known to be one of the major non-essential glycoproteins of PRV (Robbins et al., 1986b). It was shown to be homologous to glycoprotein C (gC) of HSV (Watthen & Watthen, 1986; Robbins et al., 1986a). Monoclonal antibodies against gIII are able to neutralize virus in the absence of complement (Haml et al., 1984); gIII was shown to be important also for stable adsorption of PRV to and entry into host cells (Schreurs et al., 1988), and for the efficient release of virus from infected cells (Whalley et al., 1988). Although adsorption of gIII- mutants is slower and less efficient than that of wild-type virus (Schreurs et al., 1988) these mutants are still infectious. Therefore adsorption of PRV to cells can occur by at least one other mechanism. The results from our plaque reduction and adsorption tests suggest that this mechanism cannot be affected by treatment of the virus with heparin, because concentrations up to even 500 µg/ml heparin were not sufficient to reduce virus adsorption and plaque formation below 45% and 7%, respectively, in comparison to the untreated control.

The other two spots on the gel with Mr values of approximately 105K and 56K to 71K can be identified as glycoproteins gIV and gV, respectively (Haml et al., 1984). As shown in Fig. 4, we could detect gIV on 2D PAGE analyses after immunoprecipitation of gIII using a polyclonal goat serum against a denatured Escherichia coli-produced cro–gIII fusion protein (Ryan et al., 1987). Therefore it is evident that gIV is antigenically related to gIII or to a degradation product of this protein. The polyclonal goat serum was kindly supplied by Lynn W. Enquist.

The situation about gV remains unclear, because this protein has not been characterized further nor do we know of a gene which encodes this protein. From its Mr value of about 62K, it could be speculated that gV could be identical to gp63, a PRV glycoprotein described by Petrovskis et al. (1986) which shows homology to HSV gene US7. Experiments concerning this point are in progress.

While this paper was being refereed two additional papers supporting the involvement of PRV gIII in attachment were published by Zuckermann et al. (Journal of Virology 63, 3323–3329) and Mettenleiter (Virology 171, 623–625).

We would like to thank Sabine Kaiser for her expert technical assistance and Mrs A. Hennig for preparing the photographs. Parts of this work were supported by the Hertie Foundation.

References


(Received 7 August 1989; Accepted 3 January 1990)