Plasma membrane requirements for cell fusion induced by herpes simplex virus type 1 glycoproteins gB, gD, gH and gL

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Herpes simplex virus type 1 (HSV-1) enters cells by a fusion event that occurs at neutral pH at the plasma membrane and which is mediated by virus-encoded glycoproteins (reviewed by Spear, 1993). The virus can also spread from cell to cell by inducing the fusion of cellular membranes. Glycoproteins B (gB), gD and gHL of HSV-1 are not only essential for virus entry at a post-binding stage (Cai et al., 1988; Forrester et al., 1992; Ligas & Johnson, 1988; Roop et al., 1993) and for cell–cell fusion induced by syncytial strains (Cai et al., 1988; Davis-Poynter et al., 1994), but are also necessary and sufficient for the induction of cell–cell fusion when expressed from plasmid vectors in COS 7 cells (Turner et al., 1998), and the same set of proteins from HSV-2 can cause cells to fuse in a similar transient fusion assay in the absence of any other virus components (Muggeridge, 2000).

The mechanistic details of this process remain to be defined, specifically in terms of how these four molecules interact with cell-surface receptors and with each other to mediate membrane fusion. A number of different molecules have, however, been identified that mediate the entry of alphaherpesviruses into cells by virtue of their ability to bind gD (Krummenacher et al., 1998; Nicola et al., 1998; Whitbeck et al., 1997). These are molecules of some structural diversity, including a member of the TNF/NGF receptor family (Hve A), members of the immunoglobulin superfamily, Hve B and Hve C (or nectin 2 and nectin 1, respectively), and a modified form of heparan sulphate (Shukla et al., 1999). Previous studies have addressed the requirements for some of these gD-binding proteins for both cell-to-cell spread and syncytium formation. Terry-Allison et al. (1998), showed that Hve A participates in cell–cell fusion as well as virus entry. In addition, Cocchi et al. (2000) have reported that cell-to-cell spread of wild-type strains of HSV is mediated by nectin 1, while nectin 2 works for viruses with a mutation in gD. They also concluded that syncytium formation does not involve nectin 1. Cellular receptors for gB and gHL have yet to be identified. Although our understanding of the receptor requirements for fusion remains rudimentary, it has previously been reported that cell-surface glycosaminoglycans (GAGs) may play a role in the fusion process. Shieh & Spear (1994) showed that cell fusion induced by a syncytial strain of HSV-1 is dependent on the presence of cell-surface GAGs, principally heparin sulphate, or on the addition of heparin to the medium, and it has been suggested that GAGs may alter the conformation of a viral heparin-binding protein required for the fusion event. This protein is presumably gB, since the other heparin-binding protein of HSV-1, gC, is not required for virus-induced membrane fusion (Davis-Poynter et al., 1994; Schranz et al., 1989). It has also been reported that a virus in which a lysine-rich heparin sulphate-binding domain of gB is deleted displays reduced penetration kinetics and reduced plaque size (Laquerre et al., 1998), implying a potential role in fusion for gB interactions with GAGs.

It is also unclear whether the four glycoproteins required for fusion function as a complex, although it seems unlikely that they can function independently. Cross-linking studies on HSV virions (Handler et al., 1996a) have shown the existence of very high molecular mass species containing gB, gD, gC and gHL, and these authors argued that they interact to form a functional complex. Furthermore, the cross-linking characteristics of these glycoproteins were altered during virus entry, suggesting that conformational changes may occur during fusion (Handler et al., 1996b). Nevertheless, if such a complex exists, it must be able to form in the absence of each member of the complex, since the cross-linking patterns seen with wild-type virions are unaltered in virions that lack either gB, gD or gHL (Rodger et al., 2001).

In order to examine the likely requirements for cell-surface GAGs and a gD receptor in a virus-free fusion system, we tested whether soluble heparin could inhibit fusion and whether
target cells that lacked either GAGs or a gD receptor were able to undergo fusion with the plasma membranes of COS cells expressing gB, gD and gHL. In addition, with a view to determining whether these four glycoproteins might mediate fusion as a functional complex, we tested whether gB, gD and gHL can cooperate in trans to induce polykaryocyte formation in a system that lacks any other virus components.

COS 7 cells were transfected with plasmids expressing gB, gD and gHL (as described in Turner et al., 1998) and, after 2 days, the monolayers were overlaid with Vero cells and heparin was added to the medium at concentrations ranging from 10 to 200 µg/ml. Twenty-four h later, the cells were fixed and the number of nuclei that were recruited into polykaryocytes containing 11 or more nuclei was scored. The results of three independent experiments are shown in Fig. 1. Despite some variation between experiments, we found that, at the higher concentrations tested (50–200 µg/ml), heparin reduced fusion by between 42 and 80% of the untreated control values. At the lower concentrations (10–20 µg/ml), the effect on fusion of adding soluble heparin was much less pronounced and was often negligible. One interpretation of these results is that, at high concentrations, heparin binds to gB on the surface of transfected COS 7 cells, thereby preventing its interaction with cell-surface proteoglycans on neighbouring Vero cells, and, by so doing, reduces the likelihood of membrane fusion occurring. Since it has been reported that cells lacking plasma-membrane GAGs fuse less efficiently than parental cell lines when infected with a syncytial strain of HSV-1 or when transfected with the genomic DNA of a syncytial virus (Shieh & Spear, 1994), we examined the requirement for GAGs in the virus-free fusion system by testing whether effector cells expressing gB, gD and gHL could fuse with target cells that lacked plasma-membrane proteoglycans, either as a result of metabolic depletion or as a result of a genetic defect in GAG biosynthesis.

COS 7 cells (1 x 10⁴) were seeded in 10 cm² dishes and transfected with plasmids expressing gB, gD and gHL. This seeding density was chosen to reduce the likelihood of cell–cell contact between the effector COS cells and thereby to eliminate background fusion events occurring as a result of COS–COS cell fusion. After 48 h, they were overlaid with either 1 x 10⁶ Hep2 cells that had been cultured in normal growth medium (Glasgow modified Eagle’s medium) containing 10% foetal calf serum or with 1 x 10⁶ Hep2 cells that had been cultured for 6 days in sulphate-free medium (Gibco) supplemented with 20 mM sodium chloride and 10% dialysed foetal calf serum to prevent sulphation of GAGs. This is an established method for depleting cells of sulphated GAGs (Keller et al., 1989). Twenty-four h later, the monolayers were fixed and fusion was scored by counting the number of nuclei present in syncytia containing 11 or more nuclei. The results are shown in Fig. 2(a). The two sets of Hep2 target cells fused equally efficiently with the membranes of COS 7 cells expressing gB, gD and gHL, and both sets of cells failed to fuse with the membranes of untransfected cells.

In addition to using target cells that had been depleted of GAGs by growth in sulphate-free medium, we also tested whether Chinese hamster ovary (CHO) cells with a genetic defect in GAG biosynthesis were able to undergo fusion when used as target cells in the fusion assay. In these experiments, we transfected 10⁴ COS cells with plasmids expressing gB, gD and gHL and used 10⁴ target cells in the overlay. CHO-K1 cells and derivatives of them were maintained in F-12K medium (Gibco) containing 10% foetal calf serum. CHO cells do not fuse with COS 7 cells expressing gB, gD and gHL in this transient fusion system, but the constitutive expression of one of the gD-binding herpes virus entry mediators (Hve A) in CHO cells (CHO-HveA-A12, described in Terry-Allison et al., 1998) enables them to be recruited into syncytia when overlaid onto COS 7 cells expressing gB, gD and gHL (Fig. 2b). This is consistent with the findings of Terry-Allison et al. (1998), who showed that Hve A is not only a co-receptor for virus entry, but also participates in virus-induced cell fusion. CHO cells that are genetically disabled in the ability to synthesize heparin sulphate and chondroitin sulphate, pgsA-745 (Shieh & Spear, 1994), and that express the gD receptor Hve A (pgsA-745-HveA14; a gift from P. Spear) were also examined as recipient cells in the fusion assay. These cells were not compromised significantly in their ability to fuse with transfected COS 7 cells expressing gB, gD and gHL and this result, taken together with the data shown in Fig. 2(a), suggests that the presence of cell-surface GAGs is not an absolute requirement for fusion to occur in this system, at least with the glycoproteins derived from the virus strains that we have used. This finding was somewhat surprising, in view of the fact that previous reports have shown that GAGs are required for syncytium formation.
However, it is possible that there are differences between the fusion process mediated by syncytial strains and the virus-free fusion system that we have described. There may also be differences between the amounts of Hve A expressed in the CHO-HveA cell line and those found in cells that ordinarily express Hve A, and this may perhaps bypass the need for GAGs in one assay, but not in the other.

In order to determine whether gB, gD and gHL can cooperate in trans to induce fusion, we transfected separate cultures of cells with plasmids expressing different combinations of the four glycoproteins, mixed the resulting cells and looked for evidence of fusion. COS 7 cells (10^5) were seeded in 10 cm² dishes and transfected with a total of 1 µg DNA by using Fugene reagent (Boehringer), following the protocol recommended by the manufacturer. The transfection efficiency was determined by carrying out a parallel transfection with a plasmid expressing β-galactosidase (pCDNA1.1amp lacZ; Invitrogen) and counting the proportion of cells that stained blue in the presence of X-Gal. The efficiency in this experiment was estimated to be approximately 15%. After 48 h, the transfected cells were trypsinized, mixed with an equal number of cells expressing different combinations of glycoproteins or with cells expressing β-galactosidase and replated in 20 cm² dishes. Twenty-four h later, the monolayers were fixed and the number of nuclei in syncytia containing 11 or more nuclei was counted. As shown in Fig. 3, the only set of conditions that gave rise to significant levels of fusion was when cells expressing gB, gD and gHL were mixed with cells transfected with a LacZ-expressing plasmid. All other combinations tested, namely mixing cells expressing gB and gD with cells expressing gHL, mixing cells expressing gHL and gB with cells expressing gD or mixing cells expressing gHL and gD with cells expressing gB, failed to induce fusion above the levels observed when cells expressing gB were mixed with cells expressing gD or when untransfected cells were mixed with cells expressing β-galactosidase, suggesting that all four of the glycoproteins that are necessary and sufficient to mediate fusion must be present on the same membrane and appear to act in cis during the fusion process. This is also the case in the context of virus infection, since all
four glycoproteins must be expressed on the same membrane in order for polykaryocyte formation induced by a syncytial strain of HSV-1 to occur (Davis-Poynter et al., 1994).

The results presented here, although they imply that gB, gD and gHl act in a co-ordinated and unidirectional fashion to mediate fusion, leave open the question as to what triggers the fusion reaction. It had previously been suggested (Shieh & Spear, 1994) that an interaction between gB and GAGs at the cell surface led to a conformational change in gB that triggered the fusion process. However, there appears to be no requirement for plasma-membrane GAGs in the virus-free fusion system used in this study. Nevertheless, it is clear that one of the essential requirements for HSV-1-induced fusion, both in infection and in the transfection-based fusion assay, is the interaction between gD and a gD receptor, since CHO cells that lack such a molecule are unable to fuse. The sequence of events that follows the binding of gD to one of its family of receptors, which is presumably mediated by gHl and gB and which subsequently leads to lipid mixing and fusion, remains to be elucidated.

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References


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