Identification and characterization of bovine herpesvirus-1 glycoproteins E and I

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To identify the products of the bovine herpesvirus-1 (BHV-1) gE and gI genes, we constructed baculovirus recombinants containing the putative gE or gI genes. These recombinant viruses synthesized BHV-1 gE and gI with apparent molecular masses of 84 and 41 kDa, respectively. Polyclonal antibodies against these recombinant gE or gI proteins were produced and by using these antibodies, we showed the presence of gE and gI with apparent molecular masses of 94 and 45 kDa, respectively, in purified BHV-1 virions. We also demonstrated that like their herpes simplex virus-1 and pseudorabies virus counterparts BHV-1 gE and gI form a complex. A gI mutant failed to express gI but gE was found in the virions. On the other hand, neither gE nor gI was found in the virions of a gE BHV-1 mutant. In the gI BHV-1 mutant, gI was produced but released into the medium without being integrated in the virions.

The alphaherpesviruses, which include herpes simplex viruses 1 and 2 (HSV-1 and HSV-2), varicella-zoster virus (VZV), pseudorabies virus (PRV) and bovine herpesvirus-1 (BHV-1), have a number of structurally homologous glycoproteins. Most herpesvirus glycoproteins are present in the viral envelope and are important for virus–host interactions. Glycoproteins gE and gI of HSV, VZV and PRV form a functional complex (Johnson & Feenstra, 1987; Zuckermann et al., 1988). The gE–gI complex of HSV-1, but not PRV, binds the IgG Fc fragment, which may help HSV-1 to escape from the host immune response (Bell et al., 1990; Frank & Friedman, 1989; Hanke et al., 1990; Johnson et al., 1988). Small plaques produced by gE- or gI− mutants are evidence that the gE–gI complex facilitates cell-to-cell spread of virus in cultured cells (Dingwell et al., 1994; Zsak et al., 1992). In vivo, the gE–gI complex is important for the spread of herpesviruses through the central nervous system of the host animal (Dingwell et al., 1995; Enquist et al., 1994; Kritas et al., 1994; Mulder et al., 1994). Deletion of the gE gene from both PRV and BHV-1 attenuates virulence of these viruses but maintains a strong immunizing potential and gE deletion mutants of PRV and BHV-1 are used as marker vaccines (Jacobs et al., 1993; Van Engelenburg et al., 1994; Van Oirschot et al., 1990). The nucleotide sequence of the unique short (U9) region of BHV-1 has been determined and open reading frames (ORFs) of the gE and gI genes deduced by comparison with the sequences of the previously identified gE and gI genes of HSV-1 and PRV (Leung-Tack et al., 1994).

Previously, we constructed a gE− insertion mutant of BHV-1, BHV-1/TF9-3, and a gI− insertion mutant, BHV-1/TF9-7, and found that these mutants formed smaller plaques than those produced by their parental line, IBRV(NG)dltk (Otsuka & Xuan, 1996). It is the purpose of this communication to identify the products of the gE and gI genes of BHV-1 and elucidate some of the characteristics of BHV-1 gE and gI.

The BHV-1 EcoRI C fragment was cloned at the EcoRI site of pUC19. The ORF of the gE gene was cleaved by digesting with Csp45I and the ORF of the gI gene was cleaved by digesting with Thr111I; the fragments were inserted at the Smal site of the baculovirus transfer vector, pBacPAK9 (Clontech). The transfer vectors containing the BHV-1 gE and gI ORFs were used to construct a recombinant baculovirus expressing BHV-1 gE and another recombinant expressing BHV-1 gI was constructed by the homologous recombination method described by Matsuura et al. (1987); the recombinants were designated AcBgE and AcBgI, respectively. Sf-9 cells were infected with AcBgE and AcBgI and total protein extracts of these infected cells were analysed by Western blotting using anti-BHV-1 rabbit serum. Results are shown in Fig. 1. Anti-BHV-1 serum reacted strongly with a 84 kDa protein from AcBgE-infected cells (Fig. 1A, lane 1) and with a 41 kDa protein from AcBgI-infected cells (Fig. 1A, lane 3). Anti-BHV-1 serum did not react with any band from wild-type baculovirus-infected cells or uninfected Sf-9 cells. The apparent molecular masses of gE and gI expressed by AcBgE and AcBgI are greater than those predicted from the gE or gI ORF sequences (61±2 or 39±9 kDa) (Leung-Tack et al., 1994).
indicating that these gE and gI proteins may be glycosylated like gE and gI expressed by BHV-1. Western blot analysis of tunicamycin-treated gE and gI expressed by AcBgE and AcBgI demonstrated that 84 kDa gE and 41 kDa gI were replaced by bands with apparent molecular masses of 80 and 40 kDa, respectively (Fig. 1B). This result suggests that gE and gI expressed by baculovirus recombinants contain N-linked sugars.

Anti-BgE and anti-BgI sera were produced in mice by inoculating the extracts of SF-9 cells infected with AcBgE or AcBgI and were used to study gE and gI gene products in BHV-1-infected MDBK cells and purified virions. Fig. 2 shows Western blotting analyses of BHV-1 and gE and gI mutants using the anti-BgE and anti-BgI sera. The anti-BgE serum reacted with a protein with an apparent molecular mass of 94 kDa from the extract of MDBK cells infected with IBRV(NG)dltk (Fig. 2A, lane 1). However, the anti-BgI serum did not detect any protein in the extracts of MDBK cells.
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To determine if gE and gI underwent N-glycosylation, BHV-1-infected cells were treated with tunicamycin from 0 to 48 h post-infection and the infected cell extracts were analysed by Western blotting (Fig. 2B, lane 1–4). The 94 kDa BHV-1-expressed gE was replaced by a band with an apparent molecular mass of 84 kDa. The 45 kDa BHV-1-expressed gl was replaced with a band with an apparent molecular mass of 40 kDa. These results indicate that recombinant-baculovirus-expressed gE and gl contain N-linked sugars.

In this study we have identified BHV-1 gE and gI, the respective products of BHV-1 ORFs 7 and 6, as glycoproteins with apparent molecular masses of 94 and 45 kDa, respectively. These molecular masses are larger than those predicted (61 ± 2 kDa and 39 ± 9 kDa, respectively) from the sequences of ORF 7 and 6. Since tunicamycin treatment reduced the

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To determine if gE and gI form a complex as is the case in HSV and PRV (Johnson & Feenstra, 1987; Zuckermann et al., 1988), BHV-1-infected MDBK cells were labelled with [H]$^3$glucosamine from 6 to 24 h after infection and the extracts were immunoprecipitated with anti-BgE or anti-Bgl and analysed by SDS–PAGE. As shown in Fig. 3(A), anti-BgE or anti-Bgl precipitated both gE and gl together from extracts of IBRV(NG)dltk-infected cells (lanes 1 and 5), demonstrating that gE and gl form a complex. In the gl$^{-}$ mutant (BHV-1/TF9-7), where gl is not expressed, gE appeared to be synthesized and present in the infected cell extracts without forming a gE–gl complex (Fig. 3A, lane 3). Furthermore, gE was detected in the purified virions of the gl$^{-}$ mutant (BHV-1/TF9-7) as shown in Fig. 3(B), lane 3. On the other hand, gl was detected neither in the gE$^{-}$ mutant (BHV-1/TF9-3)-infected cell extracts (Fig. 3A, lane 6) nor in the virions of the gE$^{-}$ mutant (BHV-1/TF9-3) (Fig. 3C, lane 2). However, gl was detected in the medium of the gE$^{-}$ mutant-infected cells (Fig. 3C, lane 5).

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apparent molecular mass of gE from 94 kDa to 84 kDa, it would appear that gE undergoes tunicamycin-sensitive N-linked glycosylation and some other tunicamycin-resistant modifications such as O-linked glycosylation. On the other hand, tunicamycin treatment reduced the molecular mass of gI, from 45 kDa to 40 kDa suggesting that gI contains only N-linked sugars.

Like gE and gI of other alphaherpesviruses such as HSV, VZV and PRV, these proteins in BHV-1 are involved in the cell-to-cell spread of virus since the gE− and gI− mutants of BHV-1 (BHV-1/TF9-3 and BHV-1/TF9-7, respectively) produced smaller plaques when re-infection of cells by virus released in the medium was prevented. When re-infection was allowed, the gE− and gI− mutants grew to about the same level as the parental virus suggesting that gE and gI were not involved in the process of virus maturation and release or attachment and penetration of the cells.

It has been demonstrated for several alphaherpesviruses that gE and gI can form a complex (Johnson & Feenstra, 1987; Mijines et al., 1996; Yao et al., 1993; Zuckermann et al., 1988), most likely a heterodimer (Weahly et al., 1993). gE and gI of HSV and VZV, when individually expressed in heterologous expression systems, were readily transported from the endoplasmic reticulum (ER) to the plasma membrane (Dubin et al., 1990; Litwin et al., 1992; Yao et al., 1993). In PRV, export of gE and gI from the ER was inefficient unless both proteins were present (Weahly et al., 1993). In feline herpesvirus (FHV)-infected cells, gE–gI interaction is required to allow exit of gE from the ER (Mijines et al., 1996).

Our results indicated that gE is synthesized, processed and transferred to the virus envelope in the gI− mutant of BHV-1. On the other hand, gI is synthesized in the gE− mutant but released into the medium. The apparent molecular mass of the gI released into the medium is 45 kDa, which is about the same as that of gI produced by the wild-type virus. The putative amino acid sequences of gE and gI revealed that both contained a hydrophobic anchor region at their carboxy terminus (Leung-Tack et al., 1994). It may be that mature gI lacks the hydrophobic anchor region at the carboxy terminus. In the gE− mutant, the gI precursor may be synthesized and processed as normal but then released into the medium because gI cannot be incorporated in the viral envelope without forming a complex with gE. Interestingly, gI produced by the baculovirus recombinant, which may be processed differently from gI in BHV-1-infected cells, was not released into the medium but remained associated with the membrane of infected cells (data not shown).

While this manuscript was in preparation, Whitbeck et al. (1996) identified BHV-1 gE and gI. They reported the presence of a 61.5 kDa gI precursor which is cleaved to 45 kDa and 16 kDa products. We failed to detect the 61.5 kDa gI precursor or 16 kDa product in BHV-1-infected MDBK cells. This is probably because the 61.5 kDa precursor is present at low concentration in BHV-1-infected MDBK cells and that the anti-BgI serum used was not sensitive enough to detect 61.5 kDa gI precursor or 16 kDa product in the Western blotting analyses.

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