Proteins of bovine ephemeral fever virus

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The proteins of bovine ephemeral fever virus (BEFV) were examined in purified virions and in infected BHK-21 cells. Five structural proteins were named L (180K), G (81K), N (52K), M1 (43K) and M2 (29K). The 81K G protein incorporated [3H]glucosamine, was removed from virions by treatment with Triton X-100 and bound monoclonal antibodies which were both neutralizing and protective. Treatment of virions with Triton X-100 and 0.2 to 1.0 M-NaCl progressively released L, M1 and M2. The N protein remained associated with nucleocapsids in up to 2.5 M-NaCl. The glycoprotein (G), nucleoprotein (N) and matrix protein (M2) were phosphorylated. In BEFV-infected BHK-21 cells, five virus-induced proteins were detected from 12 h post-infection. The L, N, M1 and M2 proteins corresponded to those detected in virions whereas the G protein existed in two forms. In tunicamycin-treated cells these occurred as 67K and 71K non-glycosylated precursors. In the absence of tunicamycin, 77K and 79K glycosylated forms were further modified to produce the 81K virion G protein and a 90K cell-associated form. Five viral proteins were also detected in cells infected with the closely related Berrimah virus; the Berrimah virus G protein was also present in two forms.

Introduction

Bovine ephemeral fever is an acute and disabling viral infection of cattle and water buffalo. It occurs in many tropical and subtropical regions of the world and is recognized to be of major economic importance in Australia, Japan, China and South Africa (St George & Standfast, 1988). The disease is caused by an arthropod-borne rhabdovirus, bovine ephemeral fever virus (BEFV), which appears to exist as a single serotype world-wide. Several serologically related viruses have been isolated from healthy cattle and insects but none of these has been shown to cause disease. These include Berrimah (BRMV) and Kimberley viruses from Australia, Malakal virus from Africa and Puchong virus from Asia (Liehne et al., 1981; Gard et al., 1983; Calisher et al., 1989; Walker & Cybinski, 1989). In a recent study of a large number of rhabdoviruses, serological cross-reactions were detected between viruses of the BEFV serogroup and rabies-related viruses, suggesting that these viruses form a large complex within the lyssavirus genus (Calisher et al., 1989).

Despite its economic importance, little is known of the molecular structure of BEFV. Della-Porta & Brown (1979) reported that BEFV has bullet-shaped morphology, contains an ssRNA genome and has an unusual virion structure which consists of six structural proteins including a trypsin-sensitive glycoprotein, an NS protein and two matrix proteins. We have recently described the antigenic structure and properties of the virion glycoprotein which has at least three antigenic sites containing neutralizing and protective epitopes (Cybinski et al., 1990). In this paper, we have re-examined the virion proteins of BEFV and studied protein synthesis in BEFV-infected cells. We report a simpler virion structure which resembles that of rabies virus and describe intermediates in the biosynthesis of two forms of glycoprotein found in BEFV-infected cells.

Methods

Origins of viruses. BEFV (strain BB7721) was isolated in 1968 from the blood of an infected cow at Charters Towers, Australia (Doherty et al., 1968). The virus was experimentally passaged in calves and in sucking mice before adaptation to cell culture. BRMV (strain DPP63) was isolated near Darwin, Australia in 1981 from blood taken from a healthy steer. The virus was passaged from the infected animal directly in BHK-21 cells and was shown to be distinct from BEFV by the neutralization test (Gard et al., 1983). Both viruses were plaque-cloned three times in Vero cells.

Cell culture. BHK-21 cells were grown at 37°C in RPMI 1640 medium supplemented with 14 mM-HEPES, 10% foetal calf serum, 100 units/ml penicillin and 100 μg/ml streptomycin in 850 cm² roller flasks or in 96-well plates.

Growth and radiolabelling of viruses. Confluent BHK-21 cells in roller flasks were infected at a multiplicity of 0.4 p.f.u./cell and incubated at 37°C for 1 h. The inoculum was removed and replaced with RPMI
1640 medium containing 28 mM-HEPES, 0.1% bovine serum albumin Fraction V (BSA), 100 units/ml penicillin and 100 μg/ml streptomycin (1640/BSA). For preparation of virus labelled with L-[35S]methionine, the medium was removed 10 h post-infection and replaced with methionine-free 1640/BSA medium containing 0.3 μg/ml actinomycin D. After 2 h at 37 °C, 5 μCi/ml L-[35S]methionine (Amersham) was added and the cultures were incubated at 37 °C until viruses were harvested 20 to 24 h post-infection. For preparation of virus labelled with L-[32P]orthophosphate for 2 h and harvested 14 h post-infection. Infected cell culture media were treated with 0.1 mM-PMSF at the time of harvest and stored briefly at 4 °C until required for virus purification.

**Virus purification.** Infected cell culture fluid was clarified by centrifugation at 5000 r.p.m, for 30 min (Sorvall SS34 rotor). Virus was pelleted from the supernatant fraction at 30000 r.p.m, for 90 min (Kontron TFF 45 rotor) and resuspended in a small volume of 120 mM-NaCl, 1.2 mM-EDTA, 0.1% BSA, 12 mM-Tris-HCl, pH 7.6 (NTE/BSA). The pelleted virus was applied to a 15% to 60% (w/w) sucrose gradient in NTE/BSA and centrifuged at 40000 r.p.m, for 30 min (Beckman SW41 Ti rotor). Two visible bands were detected in gradients; the upper band contained non-infectious truncated particles. The lower band, containing infectious virions, was diluted in two volumes of NTE/BSA and centrifuged at 70000 r.p.m, for 10 min (Beckman TLA 100-3 rotor). The virus pellet was resuspended in a small volume of NTE/BSA, applied to a 15% to 60% (w/w) sucrose gradient in NTE/BSA and centrifuged to equilibrium density at 40000 r.p.m, for 16 h (Kontron TST 55 rotor). The virus band was then diluted in two volumes of NTE/BSA and the virus was pelleted at 70000 r.p.m, for 10 min (Beckman TLA 100 3 rotor). For some preparations the initial rate-zonal gradient was omitted from the purification procedure.

**Detergent disruption of virus.** The procedure used was similar to that described by Zaides et al. (1979). L-[35S]Methionine-labelled virus (1 μg; approx. 2 × 10^6 d.p.m.) purified from infected cell lysate was treated at room temperature for 20 min with 0.2% to 2.0% Triton X-100 and up to 2.5 mM-NaCl in 10 mM-Tris-HCl pH 7.4 as described. The samples were then chilled on ice and layered over a cushion of 15% (w/v) sucrose in 10 mM-Tris-HCl pH 7.4 containing 0.1% BSA. The samples were then centrifuged at 70000 r.p.m, for 15 min at 4 °C (Beckman TLA 100.3 rotor). The soluble fractions were collected from above the sucrose cushion, the insoluble pellets were dissolved in an equal volume of NTE/BSA and each fraction was analysed by SDS-PAGE and fluorography.

**Preparation of infected cell extracts.** Pulse-labelling and pulse-chase experiments in infected cells were conducted using 96-well microtitre plates (Nunc) containing 1 × 10^5 BHK-21 cells/well. Wells were infected with 25 μl of virus at a multiplicity of 10 p.f.u./cell. After 1 h adsorption at 37 °C the inoculum was replaced with 100 μl 1640/BSA medium. Prior to pulse-labelling, cultures were treated with 0.3 μg/ml actinomycin D in methionine-free or glucose-free 1640/BSA medium for 3-5 h and pulse-labelling was conducted in the same medium containing 100 μCi/ml L-[35S]methionine or D-[^3]Hglucosamine. When required, 1 μg/ml tunicamycin was added to cultures 3-5 h before, and also during, the pulse-labelling period. For preparation of cell lysates, the cells were washed twice with phosphate-buffered saline and then treated with 100 μl 2% SDS, 1% 2-mercaptoethanol, 10% glycerol, 0.1 mM-PMSF, 10 mM-sodium phosphate pH 6.8, 0.001% bromophenol blue for 5 min at room temperature. Lysates were then heated at 95 °C for 2 min and stored at -20 °C until required for electrophoresis.

**Polyacrylamide gel electrophoresis.** Electrophoresis was conducted in 10% SDS-polyacrylamide gels overlaid with a 4% stacking gel prepared according to Laemml (1970). Gels were run at 100 V for 90 min in vertical mini-gels (Hoeffer) and then fixed in acetic acid/methanol/water (1:7:2) for 30 min. Gels were stained with Coomassie blue R-250 (Bio-Rad) or soaked in Amplify (Amersham) for 30 min, dried at 60 °C under vacuum and exposed to Amersham Hyperfilm-max or Kodak AR X-ray film at -70 °C. Densitometric scans of fluorograms were conducted using a GS30 Scanning Densitometer (Hoeffer) linked to an IBM AT-compatible GS356 Data Analysis System.

**Immunoblotting.** Protein immunoblotting was conducted essentially as described previously (Walker et al., 1987). Proteins were transferred from polyacrylamide gels to nitrocellulose paper by the electroblot method. The nitrocellulose was blocked with 3% gelatin and treated with monoclonal mouse IgG (1/5000) or mouse ascitic fluid (1/500), followed by biotinylated sheep anti-mouse IgG (Amersham) and biotin–streptavidin–peroxidase complex (Amersham) in 1% gelatin. Immunoblots were developed using the substrate 4-chloro-1-naphthol (Bio-Rad). BEFV-specific monoclonal antibodies DB5, 17B1, 11A3 and 2C6 were described by Cybinski et al. (1990). Fukuoka virus-specific polyclonal mouse ascitic fluid was produced in adult BALB/c mice by inoculation with virus grown in suckling mouse brain.

**Results**

**BEFV structural proteins**

BEFV was labelled with L-[35S]methionine and purified from infected BHK-21 cell lysate by rate-zonal and equilibrium density gradient centrifugation in sucrose as described. Infectious virus recovered from sucrose gradients was analysed by SDS-PAGE and fluorography. Five structural proteins (180K, 81K, 52K, 43K and 29K) were detected in purified virions by Coomassie blue staining (Fig. 1, lane 1) and by fluorography (Fig. 1, lane 2). Truncated, defective interfering (DI) particles collected from a slowly sedimenting, non-infectious peak in rate-zonal gradients also contained five viral proteins (Fig. 1, lane 3). In highly purified infectious virus preparations or in DI particle preparations, there was no evidence of a sixth structural protein as described previously (Della-Porta & Brown, 1979).

Fluorographic analysis of [^3]Hglucosamine-labelled virions purified from BEFV-infected cells indicated that only the 81K structural protein was glycosylated (Fig. 1, lane 4). The 81K protein also reacted in immunoblots when gradient-purified BEFV was reacted with neutralizing BEFV monoclonal antibodies DB5 and 17B1 (Fig. 1, lanes 5 and 6). Non-neutralizing BEFV monoclonal antibodies 11A3 and 2C6 reacted with the 52K and 29K virion proteins respectively (Fig. 1, lanes 8 and 9), confirming the viral origin of these proteins. Polyclonal mouse ascitic fluid to the unrelated lyssavirus, Fukuoka
Fig. 1. SDS-PAGE of proteins in BEFV virions and DI particles purified from infected cell culture fluid by sedimentation velocity and equilibrium density gradient centrifugation. Virion proteins stained with Coomassie blue (lane 1). Fluorograms of virion proteins labelled during infection with L-[35S]methionine (lane 2), DI particle proteins labelled during infection with L-[35S]methionine (lane 3) and virion proteins labelled during infection with D-[6-3H]glucosamine (lane 4). Immunoblots of virion proteins stained with BEFV neutralizing monoclonal IgGs DB5 (lane 5) and 17B1 (lane 6), Fukuoka virus polyclonal mouse ascitic fluid (lane 7) and BEFV non-neutralizing monoclonal ascitic fluids I1A3 (lane 8) and 2C6 (lane 9).

The structural phosphoproteins of BEFV were identified by labelling infected cells with [32P]orthophosphate. BEFV was purified from infected cell lysates and analysed by SDS-PAGE and autoradiography as described. Phosphate label was detected in the 81K glycoprotein and in the 52K and 29K structural proteins (Fig. 2, lane 2). A band of low Mr phosphate label (B) migrating ahead of the bromophenol blue marker was removed from virions by treatment with Triton X-100, as was the 81K glycoprotein (Fig. 2, lane 3). Phosphate-labelled material migrating close to the origin (A) was removed by treatment with RNase A and appeared to be viral genomic RNA (Fig. 2, lane 4).

**Detergent disruption of BEFV**

Conditions for progressive disruption of BEFV by treatment with detergent and salt were determined using L-[35S]methionine-labelled virus purified from infected cell lysate. The virus was treated with 0.2% to 2.0% Triton X-100 (ratio, 200 to 2000 µl/mg protein) in the absence of NaCl or with 0.2% Triton X-100 in the presence of 0.05 to 2.5 m-NaCl. Treatment with 0.2% Triton X-100 without salt completely solubilized the 81K glycoprotein and resulted in a clearer resolution of the 52K and 43K bands (Fig. 3b). This was due to the removal of fragments of the glycoprotein which were often detected in purified virus preparations by probing with monoclonal antibodies specific for the 81K protein (result not shown). Increasing the detergent concentration up to 2% Triton X-100 caused no further solubilization of viral proteins (Fig. 3c). Treatment of BEFV with 0.2% Triton X-100 in the presence of increasing salt concentration resulted in progressive solubilization of the 180K, 43K and 29K proteins in the range of 0.2 to 1.0 m-NaCl (Fig. 3d to h). There was no clear evidence of a differential solubility of these proteins under these experimental conditions. In the presence of 1.0 m-NaCl only the 52K protein remained associated with insoluble particles (Fig. 3h). These particles remained insoluble in salt concentrations up to 2.5 m-NaCl (not shown).

**Proteins synthesized in BEFV-infected cells**

BHK-21 cells were mock-infected or infected with BEFV at a multiplicity of 10 p.f.u./cell. At 6 h and 8 h post-infection, and at 4 h intervals thereafter, cells were labelled with L-[35S]methionine for 30 min, harvested and analysed by SDS-PAGE and fluorography. Viral protein synthesis was first detected 12 h post-infection at an early stage of cytopathic effect and was complete by 28 h post-infection (Fig. 4). Inhibition of cellular protein synthesis coincided with the maximum rate of synthesis...
of viral proteins, which occurred at 16 h post-infection. Five proteins were induced in BEFV-infected cells and these corresponded to 180K, 81K, 52K, 43K and 29K virion proteins. The glycoprotein appeared as a doublet band which migrated in gels slightly faster than the 81K virion protein (77K and 79K bands). The rates of synthesis of the viral proteins were similar to their relative proportions in virions except for the 43K protein, which appeared relatively more abundant in infected cells.

Proteins synthesized in BRMV-infected cells

In order to confirm this pattern of protein synthesis, the closely related BRMV was examined in BHK-21 cells infected at 10 p.f.u./cell. The cells were pulse-labelled with L-[35S]methionine and harvested at intervals post-infection for analysis by SDS–PAGE and fluorography as described for BEFV. BRMV was less effective in inhibiting cellular protein synthesis than BEFV at the same m.o.i. but virus-induced proteins were clearly detected from 12 h post-infection (Fig. 5). BRMV proteins apparently similar to those of BEFV were detected and had Mr's of 180K, 52K and 29K. As for BEFV, a doublet viral protein was detected in BRMV-infected cells, the lower band migrating in SDS gels slightly faster (75K) than the BEFV glycoprotein doublet. A 40K protein in BRMV-infected cells appeared to correspond to the BEFV 43K protein.

BEFV glycoprotein biosynthesis

Intracellular precursors and intermediates in the synthesis of the BEFV glycoprotein were investigated. BHK-21 cells were mock-infected or infected with BEFV at a multiplicity of 10 p.f.u./cell. At 12 h post-infection the
cells were treated with methionine-free medium containing 0.3 μg/ml actinomycin D. At 16 h post-infection the cells were pulse-labelled for 10 min with 100 μCi/ml L-[35S]methionine in the same medium and then chased with medium containing 1 mg/ml methionine. At intervals after labelling, the cells were harvested and analysed by SDS–PAGE and fluorography. The results are shown in Fig. 6. Two intracellular glycoprotein bands (77K and 79K) were detected immediately after pulse-labelling; there was little change in these bands during the first 30 min of the chase period. However, by 1 h after labelling the 77K form was absent, the 79K form was less prominent and more diffuse bands of Mr 81K and 90K had appeared. After 2 h and at later times only the 81K and 90K forms were detected. The 81K band resembled the virion glycoprotein and appeared to be removed from infected cells. The 90K glycoprotein appeared in infected cells in similar quantities to the 81K form but was not detected in BEFV virions.

To identify precursors of N-linked glycoproteins, BEFV-infected cells were pulse-labelled with L-[35S]methionine for 10 min as above in the presence of 1 μg/ml tunicamycin and chased with medium containing tunicamycin and 1 mg/ml methionine. Two glycoprotein precursors were detected and had Mr's of 67K and 71K (Fig. 7). A methionine chase in the presence of tunicamycin resulted in little change in the pattern of labelling in the two bands up to 6 h after labelling. A similar experiment was conducted in which cells were labelled for 1 h with D-[6-3H]glucosamine in the presence of tunicamycin and chased with glucose-enriched medium. There was no evidence of incorporation of label into the 67K and 71K forms. To identify accumulated viral glycoproteins, BHK-21 cells were infected with BEFV at a multiplicity of 10 p.f.u./cell and labelled for 1 h with D-[6-3H]glucosamine 16 h post-infection. After labelling, infected cells were harvested immediately (lane 3) or chased with excess glucose for 10 min, 20 min, 30 min and 60 min (lanes 3 to 7, respectively). The cells were harvested and analysed by SDS–PAGE and fluorography. Virus-induced proteins were compared with virion proteins (lane 1).
Table 1. \( M_r \)s and properties of BEFV proteins

<table>
<thead>
<tr>
<th>( M_r )</th>
<th>Functional assignment</th>
<th>Location</th>
<th>Post-translational modification</th>
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<tbody>
<tr>
<td>180000</td>
<td>L</td>
<td>Core-associated</td>
<td>–</td>
</tr>
<tr>
<td>90000</td>
<td>G(_{NS})</td>
<td>Non-structural</td>
<td>Glycosylated</td>
</tr>
<tr>
<td>81000</td>
<td>G</td>
<td>Envelope</td>
<td>Glycosylated, phosphorylated</td>
</tr>
<tr>
<td>52000</td>
<td>N</td>
<td>Core</td>
<td>Phosphorylated</td>
</tr>
<tr>
<td>43000</td>
<td>M(_1)</td>
<td>Core-associated</td>
<td>–</td>
</tr>
<tr>
<td>29000</td>
<td>M(_2)</td>
<td>Core-associated</td>
<td>Phosphorylated</td>
</tr>
</tbody>
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be identical to that detected in infected cells 1 h after pulse-labelling with L-[\( ^{35}\)S]methionine (Fig. 6), whereas the lower band appeared to contain 77K, 79K and 81K proteins immediately after the labelling period but, after a 30 min chase, only the 81K component remained. The 81K and 90K bands remained in infected cells up to 8 h after the labelling period.

Functional assignments of BEFV proteins

According to their size, relative abundance and other properties, BEFV proteins were assigned prospective functions corresponding to those of rabies virus and other rhabdoviruses (Table 1). By its \( M_r \) and association with detergent-resistant core particles, the 180K BEFV protein corresponds to the rhabdovirus polymerase (L). The 81K virion surface glycoprotein (G) is clearly identified by its binding of neutralizing monoclonal antibodies, incorporation of D-[\( ^{6}\)H]glucosamine into virions, tunicamycin sensitivity in infected cells and association with the detergent-soluble envelope fraction of purified virions. The 52K BEFV protein appears to correspond to the rabies virus nucleoprotein (N). Like the rabies virus N protein, the 52K protein is phosphorylated, associated with detergent- and salt-resistant core particles and is the most abundant component in virions and virus-infected cells. The two remaining BEFV proteins (43K and 29K) are solubilized by treatment of virions with detergent and salt and appear to be similar to the polymerase-associated protein (M\(_1\)) and matrix protein (M\(_2\)) of rabies virus. The 43K BEFV protein is in similar abundance to the 29K protein in infected cells but is less abundant in virions and appears to correspond to the rabies virus M\(_1\) protein. The 29K BEFV protein is a major virion component of similar \( M_r \) to the rabies virus matrix protein (M\(_2\)). However, unlike rabies virus, in BEFV the matrix protein (M\(_2\)) and not the M\(_1\) protein is phosphorylated. The 90K BEFV glycoprotein found in infected cells appears to be non-structural and has been designated G\(_{NS}\). No corresponding protein has been reported for other rhabdoviruses.

Discussion

The classification of BEFV as a rhabdovirus was established on the basis that it has bullet-shaped virions containing a 42S ssRNA genome and proteins of similar \( M_r \) to those of vesicular stomatitis virus (VSV) and rabies virus (Murphy et al., 1972; Della-Porta & Brown, 1979). However, it has been reported that, unlike other rhabdoviruses, BEFV contains six structural proteins, including an NS protein and two matrix proteins. This structure suggested that the virus fell into a separate genus from rabies virus and VSV (Della-Porta & Brown, 1979). A more recent study of a large panel of rhabdoviruses has identified serological relationships between those of the BEFV serogroup and those of the rabies virus serogroup (Calisher et al., 1989), suggesting that these viruses constitute a large complex within the lyssavirus genus. In this study we have re-examined the proteins of BEFV and identified a structure which is similar to that of rabies virus. Five proteins were detected in infectious BEFV virions and in BEFV-infected cells. Five virus-induced proteins were also detected in cells infected with the closely related serotype BRMV. However, BRMV has proved difficult to purify from cellular proteins and analysis of its virion structure is continuing. To assist comparisons with other viruses, the BEFV proteins were designated L (180K), G (81K), N (52K), M\(_1\) (43K) and M\(_2\) (29K) according to their size, relative abundance, detergent solubility and post-translational modifications. A more definitive assignment will be obtained from nucleotide and amino acid sequence studies which are in progress.

The estimated \( M_r \)s we obtained for BEFV proteins were at variance with those reported by Della-Porta & Brown (1979). However, the previous estimations were obtained by comparison with rabies virus and VSV proteins and without reference to primary protein standards. A close correlation with our data can be obtained by substituting more recent estimations of \( M_r \) for rabies virus and VSV proteins (Zaides et al., 1979; Wunner et al., 1985). The sixth virion protein reported by Della-Porta & Brown (1979) was a minor component which comigrated with the rabies virus M\(_1\) protein (37K) in SDS–PAGE. Although we detected proteins of similar size in some virus preparations, these were removed by further purification and were not essential for viral infectivity. No viral protein of this size was detected in BEFV-infected cells.

Three phosphoproteins (G, N and M\(_2\)) were detected in BEFV virions. Della-Porta & Brown (1979) have previously reported phosphorylation of proteins corresponding to the N and M\(_2\) proteins. We observed G protein phosphorylation to be reproducible but variable
in intensity. Evidence of G protein and N protein phosphorylation has been reported for the bat rhabdovirus, Kern Canyon virus (Sokol & Clark, 1973; Sokol et al., 1974). In rabies virus the N and M1 proteins are phosphorylated (Sokol et al., 1974; Dietzschold et al., 1979). In vesiculoviruses, phosphorylation of the NS and M proteins has been reported (Sokol et al., 1974; Imblum & Wagner, 1974). Phosphorylation of the VSV NS protein appears to have an important role in the regulation of viral transcription (Hsu et al., 1982) and it has been suggested that the rabies virus M1 protein is functionally similar (Cox et al., 1981). We detected no evidence of phosphorylation of the BEFV M1 protein.

The 81K BEFV G protein was identified by [3H]glucosamine labelling and by detergent disruption of virions. The G protein was released from virions in a relatively pure form by treatment with non-ionic detergent in low salt. Other structural proteins remained virion-associated even at high detergent/protein ratios in low salt but the L, M1 and M2 proteins were progressively released as salt was increased to 1.0 M-NaCl. Similar results have been reported for rabies virus (Zaides et al., 1979). Progressive disruption of rabies virions by increasing detergent concentrations in moderate salt has also been reported (Gyorgy et al., 1971).

The G protein was released from virions in a relatively pure form by treatment with non-ionic detergent in the absence of salt. In rabies virus, the G protein induces neutralizing antibodies and confers protection against lethal challenge (Wunner et al., 1983; Witkor et al., 1984). The BEFV G protein binds monoclonal antibodies which have been shown to be neutralizing in cell culture and which provided passive protection in mice (Cybinski et al., 1990). We have recently demonstrated that purified preparations of G protein effectively protect cattle against challenge with virulent BEFV (P. J. Walker et al., unpublished results), suggesting that the G protein may be useful as a subunit or engineered vaccine against bovine ephemeral fever.

In BEFV-infected cells two distinct forms of the G protein were detected. Non-glycosylated 67K and 71K precursors were detected in tunicamycin-treated cells. During brief radiolabelling periods in the absence of tunicamycin, 77K and 79K glycoproteins containing N-linked core oligosaccharides were formed. These were subsequently modified to 81K and 90K products which appeared to represent mature viral glycoproteins. The delay in appearance of the mature glycoproteins suggests that, as for VSV, transport to the Golgi complex is required for formation of complex oligosaccharides (Zilberstein et al., 1982). The mature BEFV 81K glycoprotein appeared to be equivalent to the virion G protein. The 90K form accumulated in infected cells in similar amounts but was not detected in virions. We have yet to establish whether this non-structural glycoprotein has a role in protective immunity.

Previous reports have indicated that the virion G protein of the CVS-rabies virus can be resolved into two forms which have identical peptide maps but differ in carbohydrate content (Dietzschold et al., 1979; Coslett et al., 1980). Tordo & Poch (1988) have detected two G mRNAs in cells infected with PV-rabies virus. Each contains the G protein coding sequence but the longer one extends some 350 nucleotides into the G-L intergenic region (Tordo et al., 1986). It has not been determined whether this transcript is translated beyond the normal G termination codon in infected cells but an alternative termination codon has been identified in a different reading frame (Tordo et al., 1986). Similar results have been reported for the ERA strain of rabies virus but in the HEP-Flury strain only the longer G mRNA is transcribed (Morimoto et al., 1989). The two forms of the BEFV G protein appear to contain different coding sequences as they react differently with monoclonal antibodies (P. J. Walker et al., unpublished results). The existence of two stable precursor polypeptides that failed to label with [3H]glucosamine in tunicamycin-treated cells suggests that the two forms are translated independently. However, peptide maps of each form will be required to establish clearly the existence of distinct structural sequences. The presence of a soft termination signal at the end of the G protein gene could provide further evidence that BEFV has a genome expression strategy similar to that of rabies virus.

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References


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