Glycoproteins of Bovine Viral Diarrhoea-Mucosal Disease Virus in Infected Bovine Cells

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SUMMARY

Bovine cell cultures infected with bovine viral diarrhoea-mucosal disease (BVD) virus were radiolabelled with L-[35S]methionine or D-[2-3H]mannose followed by analysis of the labelled polypeptides by radioimmunoprecipitation and polyacrylamide gel electrophoresis in one and two dimensions. Six glycoproteins were detected in infected cells. Two abundant species had Mr of 48K and 56K to 58K while the less abundant species had Mr of 118K, 75K, 65K and 25K. When cells were radiolabelled with L-[35S]methionine in the presence of tunicamycin 56K to 58K migrated with apparent masses of 54K (a minor species) and 48K to 50K (the major molecular species) in PAGE. Endoglycosidase F digestion of virus-induced polypeptides caused a 4K to 6K reduction in the apparent molecular mass of 56K to 58K yielding a single 52K digested product, indicating that the heterogeneity of 56K to 58K was due to differences in the oligosaccharide moieties. Tunicamycin caused a drastic reduction in the yield of infectious virus indicating that the carbohydrate moieties serve a critical role in the infectious cycle of BVD virus.

INTRODUCTION

Bovine viral diarrhoea-mucosal disease (BVD) virus is a member of the family Togaviridae, genus Pestivirus. No arthropod vector is known to participate in the biological transmission of the virus between animals and as a result the virus has been grouped along with rubella virus and other less well characterized viruses in the non-arbo togavirus group (Brinton, 1980; Horzinek, 1981). The physical and chemical properties of the virions and the infectivity of the naked virion RNA are common features of the group. As more information becomes available on the replication strategies of some of these viruses, their taxonomic status will be defined more precisely. Molecular studies on the replication of BVD virus have been few, with most studies centred on the structural proteins of the virus or on the antigens released into culture fluids (Purchio et al., 1984; Coria et al., 1983; Matthaeus, 1979; Pritchett & Zee, 1975). We reported previously on the detection of 12 viral polypeptides induced by BVD virus in infected cells (Donis & Dubovi, 1987). Here we report on the identification of virus-induced polypeptides containing N-linked oligosaccharide moieties both by direct methods and by immunoprecipitation with specific antibodies in SDS-PAGE and in two-dimensional gel electrophoresis.

METHODS

Cell cultures and virus. Foetal bovine testicle (FBT) cell cultures used between their third and fifth passage were cultured in Eagle's minimum essential medium with Earle's salts (MEME) with 10% lamb serum (Gibco). The Singer isolate of BVD virus used in these experiments was prepared as described previously (Donis & Dubovi, 1987).

Isotopic labelling and preparation of cell extracts. FBT cells cultured in 12-well plates (Flow Laboratories) for at least 48 h before use, were infected with BVD virus at an input multiplicity of 100 to 300 TCID_{50}/cell or mock-infected with medium only. Infection was carried out by rinsing the cell sheet with phosphate-buffered saline (PBS), adding the virus inoculum and adsorbing it to the cells for 1 h at 37 °C. At the end of this period the
inoculum was removed, the monolayer rinsed with PBS and overlaid with MEME containing 5% lamb serum. At various times after infection, monolayers were rinsed with PBS once, with methionine or glucose-free medium (the latter containing 0.01% fructose) once and then overlaid with precursor-free medium (Irving Scientific, Santa Ana, Calif., U.S.A.). After a starvation period of 1 h, labelling was started by the addition of D-[2-3H]mannose (radioactive concentration 1 mCi/ml, sp. act. 15 Ci/mmol) to a final concentration of 400 μCi/ml or L-[35S]methionine (radioactive concentration 12 mCi/ml, sp. act. 1330 Ci/mmol) (Amersham) to a final concentration of 200 μCi/ml, and incubated for the lengths of time at the periods after infection indicated in each figure legend. In experiments where the viral polypeptides were to be analysed without immunoprecipitation, the culture medium was made hypertonic to block protein synthesis initiation by the addition of 100 or 150 mM excess NaCl (as indicated) to the culture medium starting 20 min before labelling and kept thereafter. This procedure is referred to as hypertonic initiation block (HIB) (Nuss & Koch, 1976; Nuss et al., 1975). Tunicamycin (Sigma) was added to the appropriate cultures at 15 h post infection (p.i.) to a final concentration of 1 μg/ml. Labelling was carried out from 15-5 to 16-5 h p.i. with L-[35S]methionine. When labelling was completed the medium was aspirated and the monolayer rinsed with PBS. The cells were collected with a rubber policeman and pelleted by centrifugation for 45 s in a microcentrifuge at 2000 g. A cytoplasmic extract was prepared by adding 50 μl of TNE buffer (20 mM-Tris-HCl pH 8, 100 mM- NaCl, 5 mM-EDTA) containing 1% Triton X-100 and protease inhibitors (1 mM- l,10-phenanthroline, 50 mM- phenylmethylsulphonyl fluoride, 50 μg/ml crude ovomucoid trypsin inhibitor, 50 μg/ml crude soybean trypsin inhibitor, 50 μg/ml Aprotinin (Sigma), followed by 10 strokes of a loose fitting Teflon pestle (Kontes, Vineland, N.J., U.S.A.). Nuclei and other insoluble matter were pelleted by centrifugation at 10000 g for 2 min. The supernatant fluid (cytoplasmic extract) was aspirated and stored at -20°C until used for radioimmunoprecipitation (RIP), two-dimensional gel electrophoresis or for direct analysis by electrophoresis in vertical polyacrylamide slab gels in the presence of sodium dodecyl sulphate (SDS-PAGE).

Preparation of hyperimmune calf serum. A 2 month old bovine calf was immunized with the Singer isolate of BVD virus as described by Donis & Dubovi (1987).

Radioimmunoprecipitation and polyacrylamide gel electrophoresis. Cell extracts were radioimmunoprecipitated with hyperimmune calf serum as described previously (Donis & Dubovi, 1987). SDS-PAGE was essentially as described by Laemmli (1970).

Two-dimensional gel electrophoresis. The procedure was essentially that described by O'Farrell as non-equilibrium pH gradient electrophoresis (NEPHGE) (O'Farrell, 1975; O'Farrell et al., 1977) with minor modifications (Bravo, 1984; Perdew et al., 1983). Sample preparation was as follows: cytoplasmic extracts were dissolved in lysis buffer to achieve a final concentration of 9.8 M-urea, 2% CHAPS (3-[3-cholamidopropyl]-dimethylammonio]-l-propanesulphonate), 2% ampholytes pH 3 to 10 (LKB), 10% glycerol and 100 mM-dithiothreitol. The first dimension consisted of a 120 mm by 3 mm tube gel composed of 2.6% acrylamide (acrylamide : bisacrylamide ratio 30:1:6), 2% CHAPS, 50% ampholytes pH 3 to 10, and 9 M-urea. The anolyte was 10 mM-phosphoric acid and the catholyte 20 mM-NaOH. Samples were run from the acidic to the basic end of the pH gradient. Gels were run for 3 h at 600 V. At the end of the electrophoretic run, the gels were extruded from the tubes and stored at -20°C until the second dimension electrophoresis was carried out. For this, the thawed gels were incubated on a rocking platform with two changes of equilibration buffer (62 mM-Tris-HCl pH 6.8, 2% SDS, 100 mM-dithiothreitol) for 45 min. The gel was applied across the top of a 10% acrylamide gel and held in place with 1% agarose in equilibration buffer. Molecular mass standards have been described previously (Donis & Dubovi, 1987) and were loaded on a well carved in the agarose. Electrophoresis was carried out at constant current (15 mA per 1.5 mm thick slab) until the tracking dye reached a distance of 1 cm from the bottom of the slab. After electrophoresis the slab gels were fixed and fluorographed as described previously (Donis & Dubovi, 1987).

Endoglycosidase F digestion. The conditions of the digestion were essentially as described (Elder & Alexander, 1982; Plummer et al., 1984; Tarentino et al., 1985). Cytoplasmic extracts from infected cells labelled in the presence of hypertonic medium, containing approximately 200000 d.p.m. were made 100 mM-sodium phosphate, 50 mM-EDTA, 1% Triton X-100, 0.1% SDS, 1% 2-mercaptoethanol and the pH was adjusted to 6.1. The extract was boiled for 3 min and then divided into two aliquots. Endoglycosidase F (New England Nuclear) was added to one of them to a final concentration of 40 units/ml. The total volume of the reactions was 20 μl. Both aliquots were incubated at 37°C for 20 h. The reaction was stopped by the addition of an equal volume of double concentration electrophoresis sample buffer followed by boiling for 3 min.

The effect of tunicamycin on virus yield. FBT cells grown on 4.9 cm2 wells in 12-well cluster plates for 48 h in MEME with 5% lamb serum were rinsed and inoculated with BVD virus at an input multiplicity of 0.6 or with bovine enterovirus at an input multiplicity of 0.2 as control. After a 1 h adsorption period, the inocula were removed and the cells rinsed twice with PBS and overlaid with MEME plus 5% lamb serum containing three different concentrations of tunicamycin (0.5, 1.0 and 5.0 μg/ml) plus an untreated control. Two identical sets of plates were prepared, one to be assayed for infectivity and the other to analyse the effect of tunicamycin on protein synthesis by radiolabelling with [3H]leucine. At 15 h p.i. the medium in each well was assayed and the cell
monolayer was rinsed with PBS twice and overlaid with leucine-free MEME containing 5 μCi of L-[2,3,4,5-\(^3\)H]leucine (Amersham). After a 30 min incubation period the medium was removed, the monolayers rinsed with PBS twice, harvested and the TCA-insoluble radioactivity analysed as described (Mans & Novelli, 1961). At 20 h p.i. the cell monolayers to be assayed for infectivity were frozen at −70 °C with their overlay medium. Titration of total infectivity was carried out after thawing using FBT cells (Rossi & Kiesel, 1971).

RESULTS

Analysis by SDS-PAGE

Radioimmunoprecipitation of cell extracts labelled with D-[2-\(^3\)H]mannose indicated that there were six virus-induced glycosylated polypeptides present in cells infected with the Singer isolate of BVD virus used. The \(M_r\) of these polypeptides were 25K, 48K, 56K to 58K, 65K, 75K and 118K (Fig. 1, lanes 3 to 6). The 25K polypeptide, however, appeared as a minor species in the electropherograms of RIP assays of \([3\)H]mannose-labelled cell extracts (data not shown). The 48K and 56K to 58K glycopolypeptides were the most abundant radiochemically (Fig. 1, lane 4). In a parallel experiment using L-[\(^3\)S]methionine label followed by RIP the polypeptides

![Fig. 1](image1.jpg)

![Fig. 2](image2.jpg)

Fig. 1. Radioimmunoprecipitation of infected cell extracts. Radioimmunoprecipitation of extracts from mock-infected (lanes 1, 3, 5, 7) and infected (lanes 2, 4, 6, 8) cells labelled from 14 to 16.5 h p.i. with L-[\(^3\)S]methionine (lanes 1, 2, 7, 8) or with D-[2-\(^3\)H]mannose (lanes 3, 4, 5, 6). Lanes 5, 6, 7 and 8 are prolonged exposures of lanes 3, 4, 1 and 2 respectively. Analysis was in a 10% acrylamide SDS gel.

Fig. 2. BVD virus-induced glycoproteins labelled in the presence of HIB. D-[2-\(^3\)H]mannose labelling of infected (lanes 2 and 4) and mock-infected (lanes 1 and 3) cells from 8.5 to 11 h p.i. Lanes 1 and 2, cells were labelled under HIB (100 mM excess NaCl) and cytoplasmic extracts dissolved in sample buffer for SDS-PAGE as described in text. Lanes 3 and 4, extracts were radioimmunoprecipitated with hyperimmune serum. Electrophoresed in an 8.5% acrylamide gel.
detected had $M_r$ of 37K, 48K, 56K to 58K, 65K, 75K, 80K and 118K (Fig. 1, lanes 2 and 8). Thus, our results indicated that 37K and 80K were not $N$-glycosylated. The 25K polypeptide was not immunoprecipitated efficiently and was not readily detectable, being visualized only after prolonged exposures of the autoradiograms. To confirm the validity of the results obtained by RIP, cytoplasmic extracts were analysed directly without RIP. Cytoplasmic extracts from infected cells labelled under HIB (100 mM excess NaCl) with D-[2-$^3$H]mannose showed that the 48K and the 56K to 58K polypeptides, the two most abundant glycoproteins radiochemically, could be detected without RIP (Fig. 2, lanes 1 and 2) and that no major glycoprotein was escaping detection as a result of poor immunogenicity. These glycoproteins were not detectable in cells labelled in the absence of HIB because of the high level of incorporation of tritiated mannose into host cell glycoproteins. As we reported, HIB is necessary to reduce cellular background incorporation in order to visualize viral polypeptides without the aid of RIP in one-dimensional gels (Donis & Dubovi, 1987).

Two-dimensional gel electrophoresis

The combination of NEPHGE and SDS-PAGE allowed us to identify the two abundant glycoproteins in cells labelled under isotonic medium with D-[2-$^3$H]mannose (Fig. 3). The 56K to 58K polypeptide appeared heterogeneous both in size and charge. This complex was represented by three partially overlapping spots of different size, the largest and the smallest being slightly more basic than the intermediate sized polypeptide. The 48K polypeptide seemed to be more homogeneous with respect to size but very heterogeneous in charge with four charge species being identified. Infected cells labelled with L-[35S]methionine showed three virus-specific polypeptides: a prominent 80K polypeptide, a slightly more acidic 56K to 58K polypeptide and a 48K polypeptide with charge heterogeneity (Fig. 4). The charge heterogeneity of the 48K polypeptide was similar to the polypeptides that were labelled with mannose, except that only three spots, instead of four, were present in the L-[35S]methionine-labelled extracts, and the distribution of the radiochemical species was also different. The most prominent polypeptide present in [2-$^3$H]mannose-labelled extracts was the most basic polypeptide whereas in L-[35S]methionine-labelled extracts the second most basic polypeptide was the most abundant. The 56K to 58K polypeptide appeared in L-[35S]methionine-labelled extracts as a single spot in contrast to three spots that were detected in the 56K to 58K D-[2-$^3$H]mannose-labelled cells. This single spot appeared to align with the largest of the 56K to 58K complex labelled with mannose. The 37K polypeptide was not resolved in the two-dimensional gel; only a
**BVD virus glycoproteins**

Fig. 4. Two-dimensional gel electrophoresis of polypeptides in BVD virus-infected cell extracts. L-[\textsuperscript{35}S]methionine labelling under HIB (150 mM excess NaCl) of infected (b) and mock-infected (a) cells from 14 to 15 h p.i. Arrows indicate polypeptides used to align positions in the two gels. Second dimension separation was carried out by SDS-PAGE.

A minor spot was present in that molecular weight region on the acidic side of the slab, suggesting that it did not enter the first dimension gel correctly or that it is an unusually acidic polypeptide.

**Endoglycosidase digestion**

Endoglycosidase F digestion caused the removal of the N-linked oligosaccharides from the polypeptide backbones of the 56K to 58K polypeptide, yielding a single 52K polypeptide (Fig. 5). The 56K to 58K polypeptide which usually appeared as a broad band was clearly resolved as two distinct species in this gel. It is also evident that the size heterogeneity of the polypeptide species in the 56K to 58K polypeptide complex is the result of differences in their oligosaccharide moieties, since their deglycosylation yielded a single sharp band migrating in the SDS-PAGE gel with an apparent $M_r$ of 52K. The changes in the migration behaviour of the 48K polypeptide after endoglycosidase F treatment could not be detected due to the low proportion of 48K in the starting material.

**Tunicamycin treatment**

Radiolabelling with L-[\textsuperscript{35}S]methionine in the presence of tunicamycin caused a shift in the electrophoretic mobility of several viral polypeptides or a change in their relative abundance. The 56K to 58K glycopolypeptide was the most affected by the drug which caused it to migrate as two prominent bands of under-glycosylated polypeptides with $M_r$ of 54K and 48K to 50K (Fig. 6). No effect on the mobility of the 48K polypeptide was apparent because the under-glycosylated forms of 56K to 58K migrated in this area of the gel. The 75K polypeptide was also greatly reduced but the under-glycosylated products could not be identified. The mobilities of the 118K and 80K polypeptides were not affected. The absence of changes in 80K was expected since this is an unglycosylated polypeptide. The 118K polypeptide contains $N$-linked oligosaccharides but no alteration of its mobility was observed, which may be the result of the lack of sensitivity of the gel system used to detect small differences in the molecular masses of large polypeptides and the small amounts of oligosaccharide present in this polypeptide. Minor polypeptides of $M_r$ 42K and 36K were present in tunicamycin-treated infected cells but their origin is unclear.
Fig. 5. Deglycosylation of viral glycoproteins. Endoglycosidase F digestion of BVD-infected cell extracts labelled with L-[35S]methionine under HIB. Cells were labelled at 17 h p.i. for 1.5 h and endoglycosidase F was added to the cytoplasmic extract and incubated for 20 h at 37 °C (lane 2) or incubated without the enzyme (lane 1). The star denotes the digestion product of 56K to 58K and a polypeptide of cellular origin present in both lanes.

Fig. 6. Effect of tunicamycin on the mobility of virus-induced polypeptides. Radioimmunoprecipitation of infected (lanes 2 and 4) and mock-infected (lanes 1 and 3) cells labelled with L-[35S]methionine from 15.5 to 16 h p.i. Cells analysed in lanes 3 and 4 were treated with 1 μg/ml of tunicamycin from 15 to 16 h p.i. SDS-PAGE was carried out in a gel containing 10% acrylamide, followed by fluorography.
BVD virus glycoproteins

Fig. 7. Effect of tunicamycin on the replication of BVD virus. Infectious virus yield from (a) BVD- and (b) bovine enterovirus-infected FBT cells in the presence of different concentrations of tunicamycin (bars). The effects of the drug on protein synthesis were estimated from the incorporation of [3H]leucine into TCA-precipitable material (●) (see text for details).

The effect of tunicamycin on yield of infective virus

Tunicamycin added to cells immediately after infection caused a dramatic reduction in the yields of infectious virus (Fig. 7). Accordingly, we were interested in separating the effect of glycosylation inhibition from the inhibition of protein synthesis which tunicamycin can effect in certain cell types (Miller et al., 1980). As a control to monitor cell function we determined the yield of a non-enveloped bovine virus and also measured the incorporation of L-[2,3,4,5-3H]leucine by the cells as an indicator of the levels of protein synthesis in the cells. Addition to the cultures of tunicamycin at 0.1 μg/ml caused a reduction in the yield of infectious BVD virus of more than four orders of magnitude in a single cycle of infection (Fig. 7a). Under the same conditions the yield of bovine enterovirus was unaffected by tunicamycin (Fig. 7b). These data indicate that glycosylation inhibition was responsible for the observed BVD virus yield reduction since the cells were physiologically able to support the replication of bovine enterovirus and no significant inhibition of protein synthesis was observed in tunicamycin-treated cells.

DISCUSSION

The glycosylation status of viral polypeptides is of special interest because it suggests their likely biological function. Most viral envelopes contain glycosylated polypeptides anchored in a lipid bilayer which surrounds a nucleocapsid. All alphaviruses studied carried envelope glycoproteins E1 and E2 with M, of about 50K to 59K and E3 with M, of about 10K to 14K which may be shed into the culture medium or may remain associated with the envelope (Westaway et al., 1985a). The flavivirus envelope contains a 51K to 59K polypeptide, E1, which is usually, but not always, glycosylated and a small (approx. 8K) non-glycosylated polypeptide, M. Intracellular virus contains a glycosylated polypeptide (M, approx. 20K) which is cleaved upon virus release from the cells to yield M. Flaviviruses code for a non-structural glycoprotein (M, approx. 50K) that is present in the plasma membrane of the infected cell (Castle et al., 1986; Westaway et al., 1985b). Thus flaviviruses are somewhat unusual in that they contain a non-structural glycoprotein and some of the viruses may carry envelope polypeptides devoid of oligosaccharide moieties (Westaway et al., 1985b). The SDS–PAGE analysis of extracts of BVD virus-infected cells labelled in the presence of hypertonic medium and those labelled under isotonic conditions followed by RIP are in total agreement in that the two major N-glycosylated polypeptides present in infected cells are 48K and 56K to 58K. Less abundant species are present in extracts processed by RIP: 25K, 65K, 75K, and 118K. These polypeptides are all identifiable in L-[35S]methionine-labelled extracts, but some are apparently not immunoprecipitated efficiently. The 25K polypeptide, for example, is detected more efficiently in cells labelled
in hypertonic medium using radiolabelled amino acid precursors, and can be detected in mannose-labelled extracts after long exposures (Donis & Dubovi, 1987). Labelling experiments using D-[2-3H]mannose indicate the absence of N-linked sugar moieties in 37K and 80K.

Tunicamycin blocks the first step in the pathway of the synthesis of the oligosaccharide portion of asparagine-linked glycoproteins (Tkacz & Lampen, 1975). The drug is a mixture of 16 homologous antibiotics. Unpurified preparations of tunicamycin have been reported to inhibit overall protein synthesis significantly (Miller et al., 1980). We selected a short period of tunicamycin treatment prior to labelling to avoid the detrimental effects of protein synthesis inhibition on the efficiency of radiolabelling viral glycoproteins. Long periods of tunicamycin pretreatment are not necessary since the available pool of completed lipid-linked oligosaccharide disappears exponentially with a half life of 3 to 14 min depending on the cell type under consideration (Tenner & ScheMer, 1979). The effect of tunicamycin on the replication of most enveloped virus genera has been reported. It causes profound inhibitory effects on the assembly and morphogenesis of virions as well as on the infectivity of the virions if they are released at all (Gibson et al., 1978; Leavitt et al., 1977; Ogura et al., 1977; Pizer et al., 1980; Sabara et al., 1982; Schwarz et al., 1976; Stallcup & Fields, 1981; Stohrer & Hunter, 1979).

The most remarkable change in the electrophoretic migration pattern of the viral polypeptides synthesized in the presence of tunicamycin treatment is the increased electrophoretic mobility of 56K to 58K which migrates with apparent Mr of 48K to 50K and 54K. A possible interpretation of these changes would be that the 48K to 50K and 54K polypeptides are unglycosylated and partially glycosylated forms, respectively, of the 56K to 58K polypeptide. Assuming an average increase in the Mr of a polypeptide of 2K per N-linked oligosaccharide moiety, a total of four or five of these structures per molecule could be predicted in the 56K to 58K complex. Since the under-glycosylated 56K to 58K polypeptide is migrating in gels as a 48K to 50K species it would mask any residual 48K polypeptide. Therefore it is not known what proportion of 48K remains with unaltered mobility, if it does so at all. Two-dimensional gel analysis may resolve this point. At present, we do not know the significance of the faint 36K and 42K polypeptides. The 42K polypeptide could be the under-glycosylated form of 48K, and the 36K polypeptide the completely unglycosylated form. Alternatively these newly detected polypeptides could be cleavage products of any under-glycosylated polypeptide since glycoproteins lacking their sugar moieties tend to be more susceptible to proteolytic degradation, a fact that has been shown to result in a shortened half life of the under-glycosylated polypeptide (Schwarz & Klenz, 1974; Schwarz et al., 1976). The greatly reduced yields of infectious intra- and extracellular virus indicate that the presence of oligosaccharide moieties in the glycoproteins is critical for the replication of the virus.

No proteolytic activity was detected in the preparation of endoglycosidase F used judging from the stability of the unglycosylated polypeptides (Fig. 5). The digestion was carried out on viral polypeptides and there was no subsequent RIP, which rules out possible biases resulting from the altered immune reactivity of deglycosylated polypeptides as has been shown in other systems (Alexander & Elder, 1984; Bruck et al., 1984; Montelaro et al., 1984). The observed shift in the molecular weight of the 56K to 58K polypeptide accounts for a contribution of 4K to 6K to the observed apparent molecular weights. Assuming an average molecular weight of 2K per oligosaccharide chain, these results would suggest the presence of two and three N-linked oligosaccharide moieties in the 56K and 58K polypeptides respectively. This contrasts with the four or five sites predicted by tunicamycin experiments. This discrepancy could be the result of incomplete deglycosylation after treatment, with endoglycosidase F.

In the only available study on BVD virus-induced glycoproteins in infected cells, Purchio et al. (1984) report the results of RIP assays of infected cell extracts. Three polypeptides with Mr of 115K, 80K and 55K were reported. Using tritiated glucosamine label, these authors found that only 55K contained carbohydrate. In the present report we confirm this finding and report for the first time on the presence of five additional glycopolypeptides. Two additional polypeptides, 45K and 38K, were considered minor species by Purchio et al. (1984). In contrast, we observed that both the 45K and 38K polypeptides are abundant in infected cells; moreover, the 45K (48K in this report) contains N-linked oligosaccharide moieties as shown by tritiated
mannose incorporation. When analysing purified virions by RIP, Purchio et al. (1984) found that the three most abundant proteins (115K, 80K and 55K) were also associated with virions, suggesting that they were virion structural proteins. These data are in disagreement with an earlier report by Matthaeus (1979) who detected a different set of polypeptides in purified virus, 57K, 44K and 34K; the first two being glycosylated. Almost identical results to those reported by Matthaeus (1979) were obtained when purified preparations of hog cholera virus (a pestivirus closely related to BVD virus) were analysed by Enzmann (1982). Pritchett & Zee (1975) however, detected a set of polypeptides in purified virus that is more in agreement with the findings of Purchio et al. (1984); their sizes were 93K to 110K, 70K, 50K to 59K and 27K. Other attempts to characterize the virion structural polypeptides yielded somewhat different results: two glycosylated polypeptides with Mr of 75K and 54K were found in addition to two non-glycosylated polypeptides with a M, of 66K and 26K (Coria et al., 1983). The variance in polypeptides detected in purified virus preparations is probably the result of difficulties in virus purification. At the present time it is possible to ascribe functions to only a few of these polypeptides. There seems to be a general consensus about the existence of a glycosylated polypeptide of Mr about 55K in purified preparations of virus (A. Luukkonen-Soliman and E. J. Dubovi, unpublished results). This polypeptide, which we also found in infected cells, is one of the envelope glycoproteins (direct functional evidence will be presented elsewhere; R. O. Donis et al., unpublished results). It is still unclear which other polypeptides are part of the virion. Most other reports also found a 44K to 48K glycopolyptide in purified viral preparations which could be a second envelope component. The functional role of the 25K, 65K, 75K and 118K glycopolyptides is not clear. The findings of Coria et al. (1983) suggest that 65K and 75K are associated with the virions, while the data reported by Purchio et al. (1984) suggest that 118K is also associated with the virion. It seems unlikely that all these polypeptides reported by different groups are structural components of the virions; the obvious conclusion is that they may be virus-encoded and most likely non-structural proteins or unprocessed precursors to the structural proteins that copurify with virions.

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