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Primary Structure[1] and Post-translational Processing of the Berne Virus Peplomer Protein

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The nucleotide sequence of the peplomer (P) protein gene of Berne virus (BEV), the torovirus prototype, was determined. The gene encodes an apoprotein of 1581 amino acids with an M_r of about 178K. The open reading frame was cloned behind the T7 RNA polymerase promoter and its translation product was identified as the BEV P protein precursor by *in vivo* expression and immunoprecipitation. The deduced amino acid sequence contains a number of domains which are typical for type I membrane glycoproteins: an N-terminal signal sequence, a putative C-terminal transmembrane anchor, and a cytoplasmic tail. Eighteen potential *N*-glycosylation sites, two heptad repeat domains, and a possible "trypsin-like" cleavage site were identified. The mature P protein consists of two subunits and their electrophoretic mobility upon endoglycosidase F treatment strongly suggests that the predicted cleavage site is functional *in vivo*. The heptad repeat domains are probably involved in the generation of an intra-chain coiled-coil secondary structure; similar inter-chain interactions can play a role in P protein oligomerization. Using a sucrose gradient assay the P protein was indeed shown to form dimers. The intra- and inter-chain coiled-coil interactions may stabilize the elongated BEV peplomers.

INTRODUCTION

Berne virus (BEV) is the prototype and best-studied member of a recently described group of positive-stranded animal RNA viruses, which has been proposed to form a new virus family (the *Toroviridae;* Horzinek and Weiss, 1984; Horzinek *et al.,* 1987). Torovirions are characterized by the unique tubular morphology of their nucleocapsid and by the fact that their envelope is studded with drumstick-shaped projections, resembling the spikes of coronaviruses (Weiss *et al.,* 1983). For BEV (diameter of the disc-shaped virion 120–140 nm) these peplomers measure 20 nm in length (Weiss *et al.,* 1983). Electrophoretic analysis of virion proteins has revealed the presence of four structural polypeptides in BEV particles (Horzinek *et al.,* 1984). A heterogeneous band of M_r 75K–100K is thought to represent the peplomer (P) protein, since it is membrane-associated and is recognized by both neutralizing and hemagglutination-inhibiting monoclonal antibodies (Horzinek *et al.,* 1986; Kaeffer *et al.,* 1989).

In a previous study (Horzinek *et al.,* 1986) the *N*-glycosylation of the P protein has been described. Furthermore, it was shown that in BEV-infected cells an approximately 200K *N*-glycosylated precursor of the P protein is synthesized. In immunoprecipitations this product is recognized by monoclonal antibodies directed against the P protein. Pulse–chase experiments supported the idea that the BEV peplomers consist of post-translationally processed glycopolypeptides.

In BEV-infected cells a 3′-coterminal nested set of five viral mRNAs (approximately 25, 7, 2.1, 1.4, and 0.8 kb in length) is synthesized (Snijder *et al.,* 1990a). Except for the smallest one, these RNAs are polycistronic, but only the most 5′-situated open reading frame (ORF) of each mRNA is translated. On the basis of *in vitro* translation studies (Snijder *et al.,* 1988) BEV RNA 2 was proposed to encode the P protein precursor.

Recently, we have cloned the BEV mRNAs (Snijder *et al.,* 1990a). Nucleotide sequence analysis has revealed the presence of an ORF of 4743 nucleotides (nt) in the unique 5′ region of RNA 2. In the present paper, we demonstrate that this ORF encodes the precursor of the BEV P protein; in addition to its primary structure, an analysis of possible functional domains and data on the maturation of the P protein are presented.

MATERIALS AND METHODS

cDNA synthesis and cloning

The preparation and cloning of cDNA from poly(A)-selected RNA from BEV-infected cells has been de-

[1] Sequence data from this article have been deposited with the EMBL/GenBank Data Libraries under Accession No. X52506.

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scribed recently (Snijder *et al.*, 1990a). Clones were mapped using colony hybridization techniques and restriction enzyme analysis.

Nucleotide sequence analysis

Sequence analysis was performed as described previously (Snijder *et al.*, 1990a). Sequence data were assembled using the computer programs created by Staden (1986). Every nucleotide in the sequence was determined from at least two independent cDNA clones.

Computer analysis of the protein sequence

The deduced P protein sequence was analyzed using a hydrophobicity plot (HYDROPLOT) program based on the method of Kyte and Doolittle (1982) and a signal sequence search program (SIGSEQ) based on the method of Von Heijne (1986). Amino acid sequence similarity searches were performed using the FASTP program (Lipman and Pearson, 1985) and the entries in the NBRF data base (release 20.0). More detailed comparisons were carried out by means of the DIAGON comparison program of Staden (1982).

Reconstruction and expression of the BEV P protein gene

The BEV P protein gene was reconstructed from cDNA clones 118, 113, 115, and 106 (Fig. 1) by ligation of the following restriction fragments (numbers indicating nucleotide positions in Fig. 2): clone 118 *Dra*I–*Hpa*I (2–1200); clone 113 *Hpa*I–*Bam*HI (1200–1718); clone 115 *Bam*HI–*Eco*RI (1718–3267); clone 106 *Eco*RI–*Ba*lI (3267–4853). The reconstructed gene was cloned into the *Sma*I site of pBSXho, a pBS (Stratagene) derivative which contains an *Xho*I linker (5′ CCTCGAGG 3′) in the filled *Eco*RI site of the multiple cloning region. The *Sal*I site of pBSXho was digested and filled, and the P protein gene was isolated from pBSXho by digestion of the *Xho*I site. The gene was recloned between the *Xho*I and *Sma*I sites of plasmid pTUG31, a pAR2529 (Fuerst *et al.*, 1986) derivative which contains an extended multiple cloning region (H. Vennema *et al.*, manuscript in preparation). The resulting construct, pTP, contains the complete BEV P protein gene downstream of a T7 promoter.

Expression of the recombinant P protein gene was studied by transfection (Gorman, 1985) of HeLa cells (1×10^6 cells in a 10-cm^2 dish) with 5 μg of pTP DNA. Earlier, the cells had been infected (at an m.o.i. of 5 PFU) with recombinant vaccinia virus vTF7-3, in which the expression of the T7 polymerase gene is under the control of a vaccinia promoter (Fuerst *et al.*, 1986).

Metabolic labeling of viral proteins

Embryonic mule skin (EMS) cells were infected with the P138/72 strain of BEV at an m.o.i. of 10 as described previously (Snijder *et al.*, 1988). Labeling experiments were carried out in 10-cm^2 petri dishes. The culture supernatant was removed 15 min before labeling and replaced by methionine-free or cysteine-free medium. A 30-min pulse with 100 μCi/ml [^{35}S]methionine or [^{35}S]cysteine was performed at 10 hr postinfection (p.i.). The pulse was followed by chase periods of 0, 30, or 150 min in normal DMEM containing 2% fetal calf serum. vTF7-3 pre-infected, pTP-transfected HeLa cell cultures were labeled similarly at 14 hr p.i. (= $13\frac{1}{4}$ hr post-transfection). Cells were lysed in 300 μl TESV buffer containing 1% Triton X-100 (TX-100) as described by Vennema *et al.*, (1990).

In order to study P protein oligomerization, cells were infected as described above, labeled continuously from 10 to 16 hr p.i. with 100 μCi/ml [^{35}S]methionine, and lysed in MNT buffer containing 1% TX-100 as described by Doms *et al.*, (1988).

Immunoprecipitation and endoglycosidase treatment

Polyclonal immune sera, obtained after immunization of rabbits with purified BEV, and monoclonal antibody 6B10 (Kaeffer *et al.*, 1989) were used to precipitate the BEV P protein. Immunoprecipitations were performed as described by Vennema *et al.*, (1990).

For endoglycosidase assays viral proteins were immunoprecipitated from 100 μl of cell lysate. Subsequently, one-third of the dissolved precipitate (10 μl) was treated with endoglycosidase H as described by Vennema *et al.*, (1990). The remaining 20 μl of the sample was mixed with 40 μl endoglycosidase F buffer (50 m*M* EDTA; 1% Nonidet-P40; 1% 2-mercaptoethanol; 0.125% SDS; 100 m*M* sodium citrate, pH 6.5) and divided into two 30-μl aliquots. To one of these samples 5 mU endoglycosidase F (endo F; Boehringer-Mannheim) was added. All samples were incubated at 37°C for 16 hr and analyzed by SDS–PAGE and fluorography (Snijder *et al.*, 1988).

Oligomerization assay

The quaternary structure of the BEV P protein was studied by sucrose gradient centrifugation as described by Doms *et al.*, (1988). A total of 500 μl of cell lysate (in MNT buffer containing 1% TX-100; see above) was loaded onto an 11.5 ml linear 5–20% sucrose gradient, which was spun in an SW41 rotor (Beckman) for 12 hr, 38,000 rpm at 4°. Following a twofold dilution of the gradient fractions in TESV buffer, an

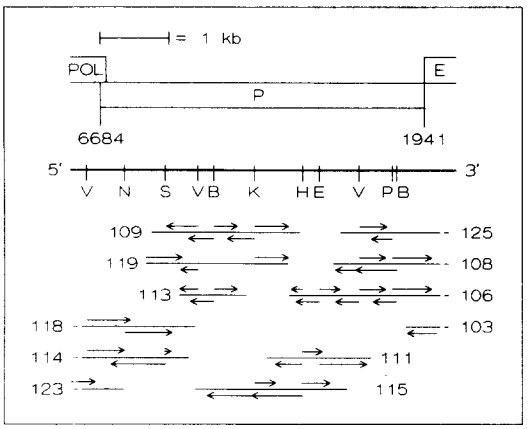

FIG. 1. Restriction map and sequencing strategy of the BEV peplomer protein gene. A map from about 2 to 7 kb upstream of the poly(A) tail is shown. Restriction sites are indicated by vertical bars and one-letter codes: B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; K, *Kpn*I; N, *Nde*I; P, *Pst*I; S, *Nsi*I; V, *Pvu*II. The position of clones used in sequence analysis is indicated; arrows indicate the sequencing direction. The P protein ORF and the flanking ORFs of the BEV polymerase (POL) and envelope (E) protein genes are represented by open boxes. The initiation and termination codons of the P protein ORF are indicated by their nucleotide distance to the start of the poly(A) tail.

anti-BEV rabbit serum was used to immunoprecipitate the viral proteins as described above. Using an identical sucrose gradient, the sedimentation behavior of the marker proteins apoferritin (from horse spleen; Pharmacia), catalase (from beef liver; Pharmacia), lactate dehydrogenase (from beef heart; Pharmacia), and albumin (from bovine serum; Pharmacia) was analyzed. Marker proteins were TCA-precipitated and analyzed directly by SDS–PAGE and Coomassie brilliant blue staining.

RESULTS

Nucleotide and amino acid sequence

We have recently described the analysis of cDNA clones covering about 10 kb (starting at the 3′ end) of the BEV genome (Snijder *et al.*, 1990a). In this nucleotide sequence a large ORF (4743 nt) was identified at positions 6684 to 1941 upstream of the poly(A) tail. The sizes of BEV RNAs 2 and 3, about 7 and 2 kb, respectively, suggested that this ORF is translated from RNA 2. Northern blot hybridizations confirmed this assumption: probes from the 5′ and 3′ ends of the ORF hybridized to RNAs 1 and 2, but did not hybridize to RNA 3; a probe from the area upstream of the ORF hybridized to RNA 1 only (Snijder *et al.*, 1990a). The position of the cDNA clones in this region and the sequencing strategy are summarized in Fig. 1.

The nucleotide and deduced amino acid sequence of the ORF and its product are presented in Fig. 2. The ORF is flanked by two short nucleotide sequences (positions 9–16 and 4813–4820) which have been shown to be conserved upstream of each ORF of BEV. They probably represent core promoter sequences involved in the transcription of the BEV subgenomic mRNAs (Snijder *et al.*, 1990a). The AUG-codon at position 61–63 is in a favorable context for use as translation initiation signal (Kozak, 1987). The ORF encodes an apoprotein of 1581 amino acids (aa) with a calculated M_r of 178,334.

A hydrophobicity plot (Fig. 3) shows that both the amino- and the carboxyl-termini of the protein are hydrophobic. An analysis of the N-terminal region using the method of Von Heijne (1986) predicts that the first 19 aa constitute a signal sequence. The somewhat larger hydrophobic stretch near the C-terminus forms a possible transmembrane anchor. Eighteen potential *N*-glycosylation sites (N–X–S or N–X–T; X may not be P) were identified (Figs. 2 and 3), which could add up to 40K to the M_r value (178K) of the apoprotein.

Using the Fastp program the amino acid sequence was compared with the entries in the NBRF protein data base, but no significant sequence similarities were observed.

Reconstruction and expression of the BEV P protein gene

To obtain experimental evidence that this large ORF encodes the BEV P protein, the full-length ORF was reconstructed from cDNA clones 118, 113, 115, and 106 and cloned downstream of the T7 promoter, resulting in construct pTP. To express the reconstructed gene *in vivo*, HeLa cells were infected with recombinant vaccinia virus vTF7-3, which expresses the T7 polymerase, and subsequently transfected with pTP DNA. Metabolic labeling revealed the synthesis of a protein of about 190K which comigrated with the P protein precursor from BEV-infected cells and which was recognized by a monoclonal antibody directed against the BEV P protein. The 190K protein was not detected in nontransfected cell lysates. These data demonstrate that the large ORF encodes the BEV peplomer protein (Fig. 4).

Cleavage of the BEV peplomer protein

The identification of a 200K precursor in infected cells and the presence of 75K–100K peplomer-specific proteins in virions (Horzinek *et al.*, 1984, 1986) indicated that the P protein is subject to a post-translational cleavage event. In the amino acid sequence of the BEV P protein a potential "trypsin-like" cleavage site consisting of five consecutive arginine residues was identified at positions 1002–1006. After the removal of a 19 aa signal sequence, cleavage between

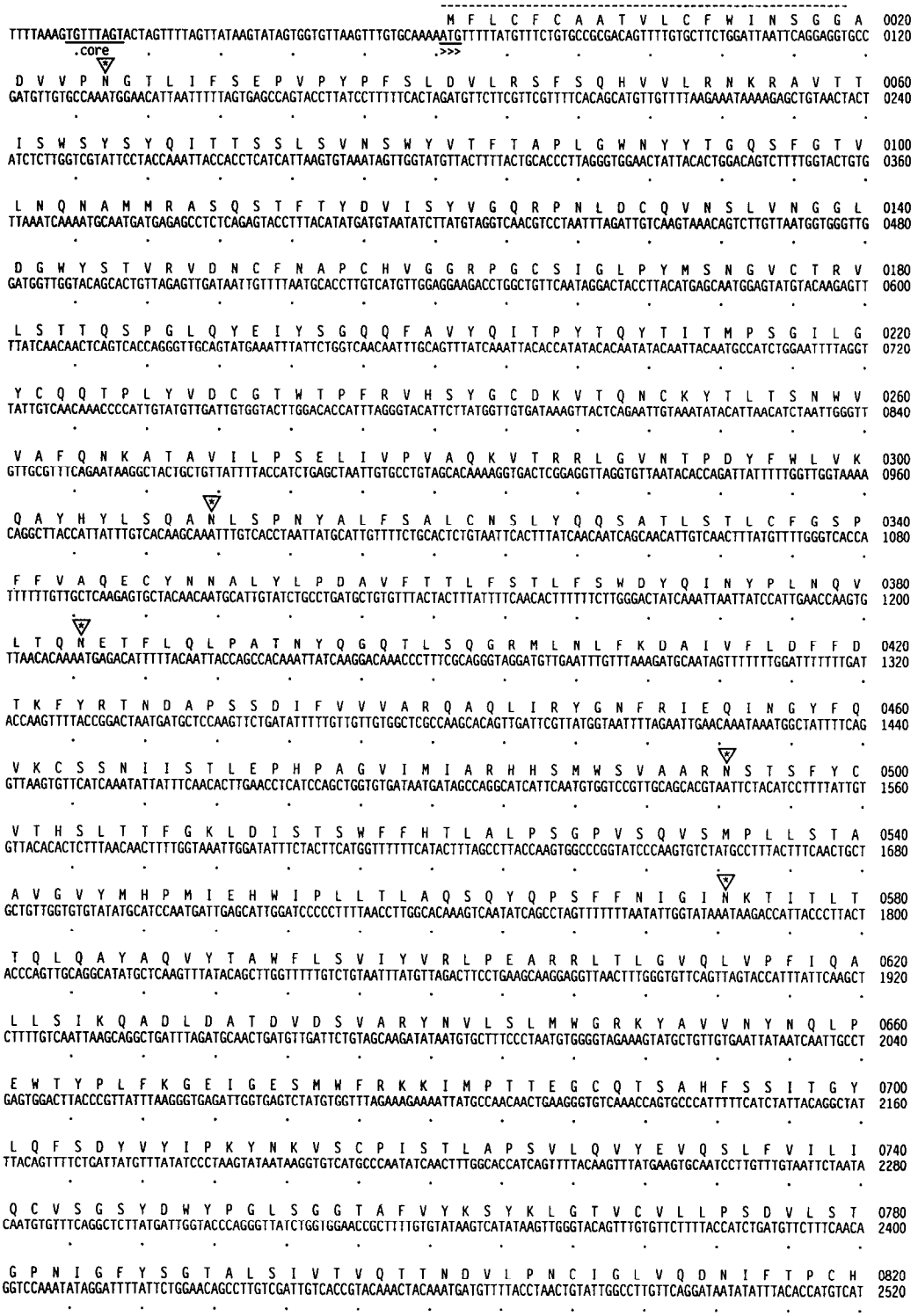

FIG. 2. Nucleotide sequence and predicted amino acid sequence of the BEV P protein gene. The 5′ and 3′ flanking sequences of the ORF, containing the postulated core promoters for RNA 2 and 3 synthesis, are also shown. Initiation (>>>) and termination (<<<) codons are indicated. Hydrophobic domains (---), heptad repeats (—), and potential *N*-glycosylation sites (triangled asterisks) are marked. The arrowhead points at the putative "trypsin-like" cleavage site, which is preceded by five arginine residues.

amino acids 1006 and 1007 would generate apoproteins of approximately 111K (987 aa; 7 potential *N*-glycosylation sites) and 65K (575 aa; 11 potential *N*-glycosylation sites).

To study the maturation of the BEV P protein precursor a pulse–chase experiment was performed. Labeled viral proteins from infected cell lysates were immunoprecipitated and treated with endoglycosidases F and

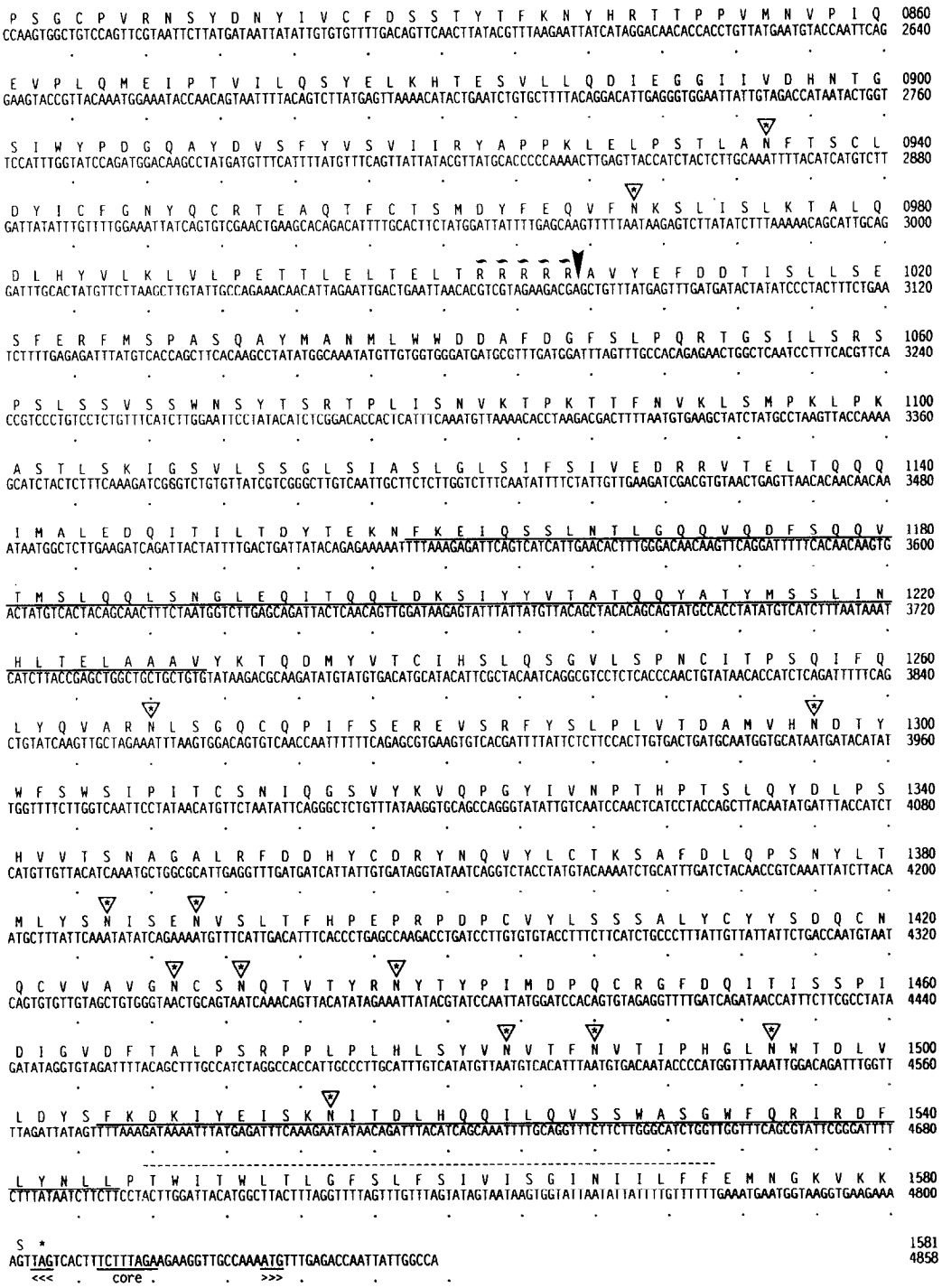

FIG. 2—*Continued*

H. Endo F cleaves glycans of both the high-mannose and complex type, leaving a single *N*-acetylglucosamine residue attached to the asparagine in the polypeptide chain (Elder and Alexander, 1982). Endo H cleaves only the high-mannose oligosaccharides which are added to the protein in the endoplasmic reticulum (ER; Tarentino and Maley, 1974). In the medial compartment of the Golgi stack these glycans are processed into endo H-resistant "complex" oligosaccharides (Dunphy and Rothman, 1985). Therefore acquisition of Endo H resistance indicates that a glycoprotein has reached the medial cisternae of the Golgi complex.

The results from the pulse–chase experiments revealed that processing of the BEV P protein precursor gave rise to the heterogeneous set of polypeptides in the range of 75K–100K which has been described be-

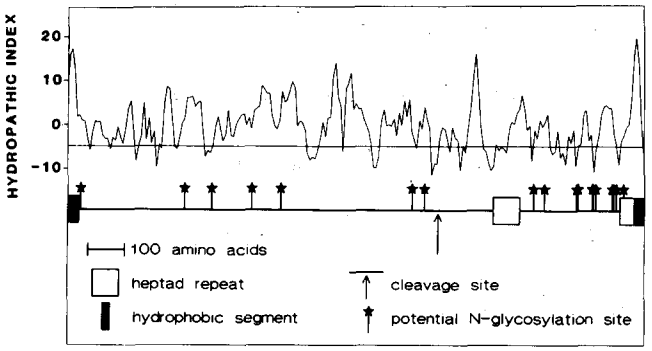

FIG. 3. Schematic representation of the position of characteristic domains in the BEV peplomer protein. The positions of heptad repeats, hydrophobic segments, potential *N*-glycosylation sites, and the putative cleavage site are indicated. In addition, a hydrophobicity plot of the amino acid sequence is presented. The plot was generated according to the method of Kyte and Doolittle (1982) using a window size of 21. The region above the line is hydrophobic.

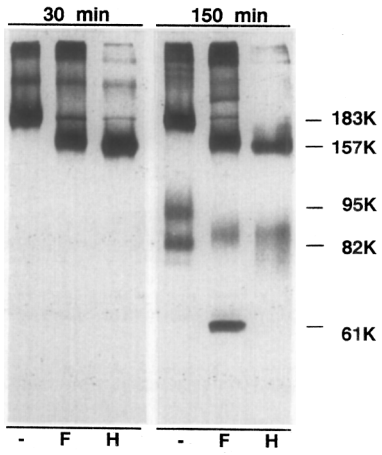

FIG. 5. Cleavage and endo F and endo H treatment of the BEV peplomer protein. Infected EMS cells were pulse labeled for 30 min with [^{35}S]cysteine at 10 hr p.i. After 30- and 150-min chase periods, the cells were lysed and viral proteins were immunoprecipitated. Samples were not treated (–) or treated with endo F (F) or endo H (H) and analyzed by SDS–PAGE. The positions of the P protein precursor (calculated at 183K) and its endoglycosidase-treated form (157K) are indicated. After a chase period of 150 min, P protein cleavage products of 95K and 82K were observed. Endo F treatment of this material resulted in the detection of a diffuse 87K band and a distinct 61K polypeptide species.

fore (Horzinek *et al.*, 1986; Fig. 5). After a 60-min chase, the first cleavage products could be detected (data not shown). In the 150-min chase material two distinct subunits of about 95K and 82K and a minor species of 77K were present. Upon endo F treatment the 82K and 77K proteins both seem to be reduced to a distinct 61K polypeptide species (Fig. 5). The size of the larger P subunit was reduced to about 87K, but in this case endo F treatment did not yield a discrete product. The oligosaccharides linked to the mature P protein were not completely endo H resistant (Fig. 5), but products in the 60K–70K range were not detected in material treated with this enzyme. We therefore assume that at least part of this material had reached the Golgi complex.

The presence of a 61K protein backbone in one of the P protein subunits supports the assumption that the stretch of five arginine residues at amino acid positions 1002–1006 functions as a cleavage site during P protein maturation. The considerable differences between predicted and calculated M_r values for the 111K subunit (and also the P protein precursor) can be explained by deviant migration. Alternatively, further processing of the N-terminal subunit of the P protein could have reduced its M_r.

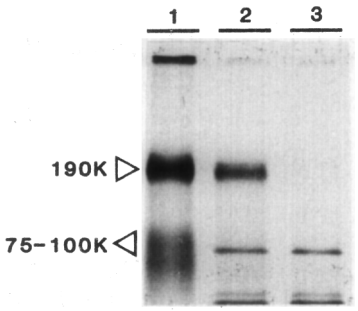

FIG. 4. *In vivo* expression of the reconstructed BEV P protein gene. Monoclonal antibody 6B10 (Kaeffer *et al.*, 1989) was used to immunoprecipitate BEV P protein from [^{35}S]methionine-labeled cell lysates. Lane 1: BEV-infected EMS cells; lane 2: pTP-transfected, vTF7-3-infected HeLa cells; lane 3: untransfected, vTF7-3-infected control cells. The pTP-expression product of about 190K is indicated.

Quaternary structure of the BEV peplomer protein

Although no significant sequence similarities between the BEV P protein and other proteins were detected (see above), a more detailed comparison with the amino acid sequence of coronaviral spike (S) proteins resulted in the identification of two interesting domains in the C-terminal part of the BEV polypeptide. At positions 1158–1230 and 1504–1545 in the predicted protein sequence a seven-residue periodicity was identified in which hydrophobic amino acids are present at every first and fourth position (Fig. 6). These so-called "heptad repeats" are indicative of a coiled-coil protein structure. In this conformation α-helical domains are stabilized by the interaction of regularly spaced hydrophobic residues which form the interface between two α-helices (Cohen and Parry, 1986).

As in the coronaviral S protein, the two heptad repeats in the BEV P protein are of unequal length, which leaves a part of the major helix unpaired. It has been

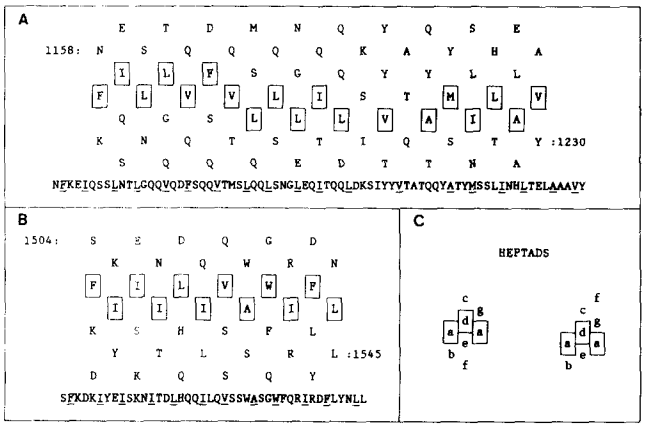

FIG. 6. Heptad repeat patterns in the sequence of the BEV peplomer protein. The sequence is listed vertically in alternating rows of three and four residues. Regularly spaced hydrophobic residues (in the a and d positions of the heptads; see panel C) are boxed. In the proposed coiled-coil structure these residues are predicted to form a hydrophobic interface, which interacts with similar regions of other α-helices. The numbers of the first and last amino acids of the large (A) and small (B) heptad repeat domains are indicated.

postulated that the major heptad repeat in the coronaviral protein is involved not only in intra-chain coiled-coiling, but also in inter-chain interactions (De Groot *et al.*, 1987), which could play a role in the previously reported oligomerization of the coronaviral S protein (Cavanagh, 1983).

The quaternary structure of the BEV P protein was studied by the sucrose gradient assay which has been employed to establish the oligomeric nature of the influenza virus hemagglutinin and the vesicular stomatitis virus G protein (Doms and Helenius, 1986; Doms *et al.*, 1988). TX-100 lysates of BEV-infected cells were subjected to centrifugation in a linear sucrose gradient to separate monomeric and oligomeric forms of the BEV P protein. A set of four sedimentation markers was used for comparison: apoferritin (an oligomer with M_r 443K; Chrichton *et al.*, 1973), catalase (a tetramer with M_r 232K; Schroeder *et al.*, 1969), lactate dehydrogenase (a tetramer with M_r 137K; Huston *et al.*, 1972), and albumin (M_r 67K; Squire *et al.*, 1968).

After gradient fractionation and immunoprecipitation, most of the P protein precursor was recovered from fraction 7 (Fig. 7), sedimenting slightly slower than ferritin (443K; fraction 6) and considerably faster than catalase (232K; fraction 11). These experiments clearly show that most of the precursor in infected cells is present in a dimeric form. Gradient fraction 7 also contained the characteristic heterogeneous peplomer material of 75K–100K which is present in mature virions. A small amount of monomeric precursor was recovered from fractions 13 and 14. The M_r of this material was slightly higher than the M_r of the precursor in the dimer peak. In addition, fractions 12 to 14 contained two bands of cleaved and possibly immature peplomer material in the 75K–100K region. On the basis of their sedimentation behavior, it can be concluded that these proteins are also in a dimeric state. Since the peaks of the 75K and the 100K species do not coincide, we assume that this material consists of homodimers of C- and N-terminal P protein subunits, respectively.

DISCUSSION

In this paper we report the identification and sequence of the BEV peplomer protein gene. An ORF of 4.7 kb which is located at the 5′ end of mRNA 2 encodes a 178K precursor protein. Antibodies directed against the virion P protein specifically immunoprecipitated a 190K protein from lysates of cells which were transfected with a T7 expression vector containing the full-length 4.7-kb ORF. The P protein displays several features which are common to membrane-associated proteins. An N-terminal signal sequence, a possible C-terminal transmembrane anchor, and a number of potential *N*-glycosylation sites were identified in the deduced amino acid sequence. Furthermore, a candidate cleavage site in the P protein precursor was identified. A host-dependent "trypsin-like" cleavage occurs during the maturation of the envelope proteins of viruses in a number of families. The cleavage site usually is a domain containing multiple basic amino acid residues (Cavanagh *et al.*, 1986). Removal of oligosaccharides (Fig. 5) from the mature P protein showed that the C-terminal P protein subunit contains a polypeptide backbone of the size predicted from the putative cleavage site.

Electron microscopy has revealed projections on the BEV surface, which consist of a globular structure on top of an elongated stem (Weiss *et al.*, 1983). A coiled-coil supersecondary structure, as it has been found for other viral (Wilson *et al.*, 1981; De Groot *et al.*, 1987) and non-viral (Metcalf *et al.*, 1987) surface proteins, can explain the elongated shape of the BEV peplomer. For the two heptad repeat domains in the C-terminal subunit (Fig. 6) α-helices of about 10 and 6 nm are predicted. In an intra-chain coiled-coil 4 nm of the major helix would remain unpaired; as postulated for its coronaviral equivalent (De Groot *et al.*, 1987), the stability of an oligomeric peplomer complex could be enhanced by the formation of inter-chain coiled-coils.

The oligomerization assay described in this paper proves that the BEV P protein in infected cells is predominantly present in a dimeric form (Fig. 7). Similar experiments in our laboratory have confirmed the dimeric nature of the coronaviral S protein (P. J. M. Rottier *et al.*, personal communication). Heptad repeats are

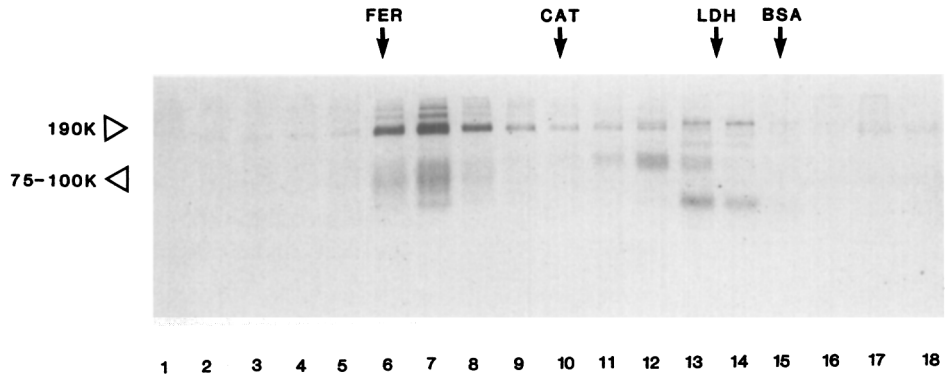

FIG. 7. Analysis of BEV P protein oligomerization. TX-100-lysed BEV-infected EMS cells were subjected to centrifugation in a 5–20% linear sucrose gradient. After fractionation from the bottom, the BEV P protein was immunoprecipitated with a rabbit antiserum. A set of four sedimentation markers was included for comparison; their position in the gradient is indicated: FER, apoferritin, M_r 443K; CAT, catalase, M_r 232K; LDH, lactate dehydrogenase, M_r 137K; BSA, bovine serum albumin, M_r 67K. Most P protein material was precipitated from fraction 7, corresponding to an M_r of about 400K. This illustrates the dimeric nature of the BEV P protein precursor in infected cells.

probably not the only domains which are involved in oligomerization, but the predicted intra- and inter-chain interactions are likely to stabilize the characteristic surface projections of both toro- and coronaviruses.

Intracellular transport from ER to medial Golgi and cleavage of the BEV P protein in infected cells seem to be quite slow: after a $3\frac{1}{2}$-hr chase, it was estimated that 40–50% of the label incorporated into the P protein was still present in uncleaved endo H-sensitive precursor material. Preliminary data on the processing of the recombinant P protein indicate that its maturation may be even slower in the absence of virus assembly: after a 5-hr chase no P protein cleavage products could be detected in pTP-transfected Hela cells (data not shown). Recently, the transport of the coronaviral S protein has been shown to be significantly slower in the absence of virus assembly: in coronavirus-infected cells the S protein acquires endo H-resistance more rapidly than in cells expressing only the recombinant S protein (De Groot *et al.,* 1989; Vennema *et al.,* 1990). Like coronaviruses, toroviruses have been shown to bud intracellularly, predominantly at the membranes of the Golgi system (Weiss and Horzinek, 1986; Fagerland *et al.,* 1986). Retardation of the BEV P protein in premedial Golgi compartments may therefore promote efficient virus budding (Vennema *et al.,* 1990).

The BEV P protein resembles the coronaviral S protein not only because of the kinetics of its intracellular transport. Both are *N*-glycosylated proteins of about the same size which contain a "trypsin-like" cleavage site (not in all coronaviruses), heptad repeats, and hydrophobic domains in comparable positions in the sequence. In addition, the electron microscopical image and the formation of dimers indicate that toro- and coronaviral spikes are also likely to have similar tertiary and quaternary structures. Within the coronavirus family the amino acid sequence of especially the C-terminal half of the spike protein is highly conserved (De Groot *et al.,* 1987). Since no significant sequence similarities between BEV P and coronaviral S protein sequences were identified, convergent evolution could be invoked to explain the comparable organization and structure of the BEV P protein. However, the recent discovery of a number of conserved domains in the polymerase genes of the coronaviruses and the torovirus BEV indicates that both virus groups are evolutionarily related (Snijder *et al.,* 1990b). Hence, we propose that the toro- and coronaviral spikes are homologous and not analogous structures. The absence of antigenic relationships and amino acid homologies among their surface proteins is indicative for the large evolutionary distance between both virus groups.

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