Sequence and N-terminal Processing of the Transmembrane Protein E1 of the Coronavirus Transmissible Gastroenteritis Virus

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SUMMARY

Sequencing of part of a clone from a transmissible gastroenteritis virus genome cDNA library led to the identification of the gene encoding the E1 matrix protein. The amino acid sequence of the primary translation product predicts a polypeptide of 262 residues which shares many features with the previously characterized murine hepatitis virus and infectious bronchitis virus E1 proteins. However, N-terminal amino acid sequencing revealed that a putative signal peptide of 17 residues was absent in the virion-associated polypeptide. The predicted mol. wt. of the mature unglycosylated product, 27800, is in agreement with the experimental Mr value.

INTRODUCTION

Transmissible gastroenteritis (TGE) is a highly contagious disease of pigs causing high mortality in neonates. The causal agent (TGE virus, TGEV) belongs to the family Coronaviridae, a group of enveloped viruses with a large, positive-stranded RNA genome (for review, see Siddell et al., 1983). Coronavirus-encoded information is expressed in the cell through a nested set of subgenomic mRNAs with common 3'-terminal sequences. The coding part of each mRNA corresponds approximately to the 5'-terminal sequences that are absent in the next smaller species. Mouse hepatitis virus (MHV) and infectious bronchitis virus (IBV) mRNAs contain at the 5' end a short non-coding sequence joined to the body sequences by discontinuous transcription; a consensus sequence identified at each intergenic region may act as a binding site for the RNA polymerase–leader complex (Spaan et al., 1983; Brown et al., 1984; Lai et al., 1984; Budzilowicz et al., 1985).

TGEV contains three major structural polypeptides: the peplomer glycoprotein E2 (200K to 220K), which forms the distinctive surface projections, the transmembrane or matrix protein E1 (29K ± 1K), and the nucleoprotein N (47K ± 1K), in which a single infectious RNA molecule of 20 kb or more is embedded (Garwes et al., 1976; Lau et al., 1986; Brian et al., 1980). In TGEV-infected cells, five species of subgenomic mRNA have been characterized, in vitro translation of which has allowed partial coding assignment (Hu et al., 1984; Jacobs et al., 1986; Rasschaert et al., 1987).

The matrix protein has been the subject of intensive studies in other coronaviruses. The N-terminal regions of E1 proteins of MHV and bovine coronavirus (BCV) bear sugar chains O-linked to Ser or Thr residues (Niemann & Klenk, 1981; Niemann et al., 1984), an unusual feature among viral glycoproteins. Also, unlike the majority of integral membrane proteins, both MHV and IBV E1 proteins lack a cleaved signal peptide (Rottier et al., 1984; Stern & Sefton, 1982a). The restriction of E1 to internal membranes seems to determine the assembly of the coronavirus particles in the lumen of the endoplasmic reticulum (Holmes et al., 1981; Tooze et al., 1985). A model of the membrane topology of E1 has recently emerged from a combination of biochemical data and analysis of its primary structure (Armstrong et al., 1984; Rottier et al.,...
1986). The amino half is formed of a transmembrane helix with two hairpin structures, whereas the carboxy half is closely adjacent to the inner surface of the viral envelope and is largely resistant to proteolysis. At each end, two short hydrophilic segments are assumed to project from either side of the membrane.

In an attempt to elucidate the functional domains of the TGEV surface glycoproteins, a cDNA library of the TGEV genome has been created in this laboratory. In this paper, we report the sequence of part of a clone encoding the E1 gene, present information about the N-terminal processing of its product and compare the features predicted from its primary structure with those of the other E1 coronavirus proteins.

**METHODS**

**cDNA cloning.** The preparation of cDNA clones will be described in more detail in a subsequent communication. cDNA was obtained from purified genomic RNA (TGEV Purdue-115 strain) using oligo-(T)$_{12-18}$ (Pharmacia) as primer and reverse transcriptase (Stiehelin, Basel, Switzerland). RNase T2-treated cDNA–RNA hybrids were dC-tailed and inserted in PstI-cut dG-tailed pBR322 (Bethesda Research Laboratories) (Zain et al., 1979; Van der Werf et al., 1981). *Escherichia coli* RR1 cells were transfected with this material. An insert (5 kb) covering the complete E1 coding region was located in the clone pTG2.15.

**DNA sequencing.** Sonicated fragments of pTG2.15 were subcloned into Smal-cut M13mp18 vector (Deininger, 1983). Sequencing was performed by Sanger’s dyeoxy technique using [$^{35}$S]dATP (New England Nuclear) as the label and the reaction products were analysed in buffer gradient gels. Each strand of DNA was sequenced at least three times.

**Sequence analysis.** Sequences were analysed using the program of Queen & Korn (1984), marketed as part of the Microgenie program (March 1985 version, Beckman), developed for the IBM PC-XT microcomputer. The program, utilizing the hydrophilicity values of Hopp & Woods (1981), the mean fractional area exposed values of Rose et al. (1985), and the flexibility values (predicted Bnorm. data) of Karplus & Schulz (1985) was written in Apple Basic (F. Borras-Cuesta & H. Laude, unpublished data).

Isolation of the E1 polypeptide and partial amino acid analysis. Purified virion polypeptides were resolved by SDS-PAGE as described by Laude et al. (1986). After localization by gel slice staining, the protein band was excised from the gel and placed in an electrodialysis chamber (Isco). The protein was eluted for 24 h in 50 mM-NaHCO$_3$ + 0.1% SDS. The reservoir buffer was subsequently changed to 10 mM-NaHCO$_3$ + 0.01% SDS and electrodialysis was performed overnight (Hunkapiller et al., 1983). About 100 pmol of protein was subjected to N-terminal amino acid sequencing. Sequential Edman degradation was done on a ‘gas phase’ Applied Biosystems 470A apparatus with its dedicated on-line PTH amino acid analyser 120A.

**RESULTS**

A long open reading frame (ORF) of 867 bases yielding a protein with the properties of E1, was identified on clone pTG2.15 of TGEV cDNA. The 5’ end of this ORF mapped at 2.48 kb from the 3’ end of the genome (Rasschaert et al., 1987). According to its length and position, the E1 ORF corresponded to the ‘unique’ region of the mRNA 5 within the set of viral RNAs characterized by Northern blot analysis (data not shown).

Inspection of the nucleotide sequence displayed in Fig. 1 revealed the presence, near each extremity, of two AACTAAAC sequences, which were assumed to be the start of the mRNA transcripts 5 (E1-encoding) and 6 (N-encoding) respectively. Therefore, although the ORF extended 23 codons upstream from the first consensus sequence, it was postulated that initiation of translation on mRNA 5 should occur downstream at either of the two proximal ATGs available. The ATG adjacent to the consensus sequence is followed by a characteristically hydrophobic stretch of amino acids, which may possibly act as a signal peptide for the translocation of E1. Alternatively, translation might start at the next ATG codon (position 184), yielding a product of 241 residues, still slightly larger in size than the matrix proteins of other coronaviruses. However, the second ATG lies in a less favourable context than the first for initiation of translation (Kozak, 1983).

Partial microsequencing of the mature E1 polypeptide was performed to confirm the site of translation initiation (Fig. 2). The N-terminal residues thus identified were found in perfect agreement with the predicted sequence up to position 14, beyond which the sequencing process was perturbed. This led us to conclude that: (i) translation of E1 cannot be initiated at the second ATG codon (position 184) and (ii) a hydrophobic peptide specified by the first 17 codons of the gene was not present in virion-associated E1.
TGEV E1 protein sequence

Fig. 1. Part of the sequence (957 bases) from the TGEV genomic cDNA clone pTG2.15. The main ORF, encoding the E1 protein, and part of the ORF corresponding to the adjacent nucleocapsid gene (broken line) are translated in the three letter amino acid code. The two consensus sequences are boxed. Proximal ATG codons are underlined, stop codons are overlined. Dots beneath Asn residues indicate N-glycosylation signals (Asn-X-Ser or Asn-X-Thr). The signal peptide-like sequence is underlined.

Fig. 2. Proximal amino acid data of TGEV E1 polypeptide. (a) Residues identified by partial N-terminal microsequencing of the virion protein are aligned over the predicted sequence of the E1 precursor. The uncharged region of the putative signal peptide is boxed. The arrow indicates the proposed site of entry of E1 into the lipid membrane. (b) Quantity of PTH derivative measured for the first 14 residues. Symbols: c, Cys suspected as no PTH derivative was detected; u, residue undetermined; ●, as in Fig. 1.
DISCUSSION

A cDNA cloned from TGEV genomic RNA was sequenced in the region corresponding to the coding part of mRNA 5 (Fig. 1). A single long ORF was found, which is shown to direct the synthesis of a coronavirus matrix-like protein. Only one additional ORF of more than 20 amino acids was detected, which was 43 residues long and within the E1 gene (position 240). Partial microsequencing of the virion-associated E1 polypeptide unambiguously established that the first residue was the Arg specified by the CGC codon at position 172 in Fig. 1. Hence it can be deduced that the single ATG codon available between the CGC codon and the upstream consensus sequence CTAAAC should be the functional initiation codon of mRNA 5. It predicts a primary translation product of 262 amino acids with mol. wt. 29.6 K, slightly higher than the Mr 25K reported for the in vitro translation product of mRNA 5 (Jacobs et al., 1986; referred to as mRNA 6 in their study).

The first 17 residues predicted from the nucleotide sequence were found to be lacking in the mature protein (Fig. 2). This oligopeptide actually fulfils the criteria of an eukaryotic signal peptide, namely a net charge immediately after the N-terminus and high degree of hydrophobicity of the 14 residue long uncharged region (McGeoch, 1985; Von Heijne, 1986). A striking feature is the presence of a triple Ala-Cys repeat, which also occurs in the signal peptide of rat oxytocin (see McGeoch, 1985). Also, the cleavage site appeared to be located between Gly and Arg, as predicted by the 75 to 80% accurate weight-matrix approach of Von Heijne (1986).

These results indicate that TGEV E1 matures through the removal of a 17 amino acid leader peptide. Accordingly, the final product is 245 amino acids long, and has a predicted mol. wt. of 27780 in the unglycosylated form; it is basic (with five net charges at neutral pH) and 44% of the residues are hydrophobic. This finding is in contrast with that reported for the matrix proteins of two other coronaviruses, MHV and IBV (Rottier et al., 1986; Armstrong et al., 1984; Stern & Sefton, 1982a; Boursnell et al., 1984). It has been proposed that the matrix proteins of the latter are inserted into the membrane by the recognition of an internal transmembrane region as a signal sequence (Rottier et al., 1985). Incidentally, in the IBV E1 sequence (Boursnell et al., 1984), the 22 in-frame codons between the consensus sequence and the initiation codon predict numerous hydrophobic residues, which might be the remnant of an ancestral signal peptide.

Pairwise comparisons of the gene sequence of TGEV E1 with those of MHV and IBV at the DNA level revealed no significant homology. The amino acid sequences showed, in contrast, a remarkable homology. The homologies found by Dayoff's optimal alignment are 38% (TGEV-MHV), 30% (MHV-IBV) and 27% (TGEV-IBV). The main regions of homology are shown in Fig. 3. An eight amino acid section is perfectly conserved among the three viruses (residues 128 to 135, TGEV). Of the three potential membrane-spanning regions (thickly underlined), the second is well conserved within the MHV-IBV pair, and the third within the TGEV-MHV pair; only the first shows a nearly equal degree of homology within both pairs. This might be indicative of functional differentiation between the three segments.

The above findings suggested that the topology of TGEV E1 within the membrane might be essentially similar to that proposed for MHV and IBV (Armstrong et al., 1984; Boursnell et al., 1984; Rottier et al., 1986). This is supported by the data presented in Fig. 4, where each profile corresponds to a computer-assisted prediction of the local tendency of the TGEV E1 polypeptide chain to hydrophilicity (Hopp & Woods, 1981), accessibility (Rose et al., 1985) or mobility (Karplus & Schulz, 1985). By combining our data with those cited above, five regions can be delineated from the amino to the carboxy end: a signal peptide (-17 to -1), an exposed glycosylated segment (1 to 29), three lipid bilayer-incorporated segments (30 to 55, 66 to 86, 98 to 117), an amphiphilic C-terminal half supposedly associated to the cytoplasmic face of the membrane (118 to 228) and a protruding C terminus.

Unlike the MHV and BCV matrix proteins, glycosylation of TGEV E1 has been reported to be of the N-linked type (Garwes et al., 1984; Jacobs et al., 1986), as for IBV (Stern & Sefton, 1982b). Indeed, two potential N-glycosylation sites are available near the N terminus of the sequence (Fig. 2). The accessibility of the second site (Asn, 38) in the lumen of the endoplasmic reticulum is uncertain since the first putative membrane-spanning segment of TGEV E1 could extend virtually up to position 30, i.e. seven residues farther than the WNFS sequence, where
### TGEV E1 protein sequence

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<tr>
<th>TGEV</th>
<th>MHV</th>
<th>IBV</th>
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<td>N P E T N C T L D F E Q S V Q L Y K E Y N L F I</td>
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Fig. 3. Comparison of the amino acid sequences (single letter code) of the E1 polypeptides of TGEV, MHV (strain A59: Armstrong et al., 1984) and IBV E1 (Beaudette strain: Boursnell et al., 1984). Dots denote a match between two residues; matches between the TGEV and the IBV sequences are indicated beneath the latter. With the simple alignment used, the homology within each E1 pair is 23% (TGEV-MHV), 26% (MHV-IBV) and 15% (TGEV-IBV). Boxed regions show homologies >60% between two or three sequences. Potential glycosylation sites are underlined. Thick bars indicate the three putative membrane-spanning segments (see text).

Fig. 4. Graphical output of three prediction methods applied to the amino acid sequence of TGEV E1 precursor polypeptide. (a) Hydrophilicity profile with a running average taken over a hexapeptide. (b) Exposure profile averaged as in (a); the straight line represents the mean area exposed calculated for the whole E1 chain. (c) Flexibility profile, with calculations made on a window of seven residues.
the MHV spanning segment is assumed to end (see Fig. 3). In contrast the side chain of Asn at residue 15 may be linked to an oligosaccharide residue, as suggested by the disturbance observed at this point during the N-terminal sequencing (see Fig. 2). The supposition that only the first N-glycosylation site is functional leads to an estimated mol. wt. of 29.5K to 30K, a value close to the Mr value of the E1 major species determined by electrophoresis (since the Mr, of a carbohydrate-rich mannose chain is about 2K; Klenk & Rott, 1980). Minor E1 species consistently observed as bands migrating more slowly in SDS–PAGE (Garwes & Pocock, 1975; Hu et al., 1984; Laude et al., 1986; Jacobs et al., 1986) might reflect a heterogeneity in the oligosaccharide chain rather than in the polypeptide chain (for example an oversized E1 polypeptide produced from mRNA 4, only 10% larger than mRNA 5). This viewpoint is supported by the fact that TGEV E1 yielded a single band after endoglycosidase H treatment (Hu et al., 1984; B. Delmas & H. Laude, unpublished results).

To sum up, the E1 protein of TGEV shares many structural features with those of MHV and IBV. It is becoming clear, however, that a certain diversity may exist despite the constraints that are necessary to achieve the distinctive architecture of a coronavirus particle. This is true at least for the small hydrophilic region protruding out of the particle, to which no biological function has been assigned so far. Our results provide substantial evidence that TGEV E1 undergoes N-terminal processing and that its exposed NH$_2$ extremity may be significantly larger and possibly more complex in secondary structure than those of IBV and MHV. Protease digestion has been shown to remove an external glycopeptide of nine residues (IBV; Cavanagh et al., 1986) and 2.5K (MHV; Rottier et al., 1984). In comparison, the TGEV E1 external segment may approach 30 residues, including four cysteines (see Fig. 2 a and 3). Recent investigations have suggested an involvement of TGEV E1 in the induction of interferon $\alpha$ from non-immune lymphocytes (see Rasschaert et al., 1987; B. Charley & H. Laude, unpublished observation). An interesting possibility is raised that this previously unrecognized activity of E1 might proceed through the interaction of its NH$_2$ free tail with the lymphocyte surface. Additional experiments are currently underway in order to study this question.

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REFERENCES


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