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# The Predicted Primary Structure of the Peplomer Protein E2 of the Porcine Coronavirus Transmissible Gastroenteritis Virus

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## SUMMARY

The complete nucleotide sequence of cloned cDNAs containing the E2 glycoproteinencoding region of the genome of transmissible gastroenteritis virus (TGEV) has been determined. A single large translatable frame of 4.3 kb starting at 8.2 kb from the 3' end of the genome was identified. Its deduced amino acid sequence contains the characteristic features of a coronavirus peplomer protein: (i) the precursor polypeptide of TGEV E2 is 1447 residues long (i.e. 285 longer than the avian infectious bronchitis coronavirus spike protein); (ii) partial N-terminal sequencing demonstrated that a putative secretory signal sequence of 16 amino acids is absent in the virion-associated protein; (iii) the predicted mol. wt. of the apoprotein is 158K; most of the 32 potential N-glycosylation sites available in the sequence are presumed to be functional to account for the difference between this and the experimentally determined value (200K to 220K); (iv) a typical hydrophobic sequence near the C terminus is likely to be responsible for anchoring the peplomer to the virion envelope.

## INTRODUCTION

Transmissible gastroenteritis virus (TGEV), a highly enteropathogenic virus of pigs, belongs to the family Coronaviridae, a group of enveloped viruses with a large, positive-stranded RNA genome (see Siddell et al., 1983). Studies on the organization and expression of the TGEV genome (Dennis & Brian, 1982; Hu et al., 1984; Jacobs et al., 1986; Kapke & Brian, 1986; Rasschaert et al., 1987) tend to confirm the findings reported for two other members of the Coronaviridae, murine hepatitis (MH) and avian infectious bronchitis (IB) viruses (see Laude et al., 1987). Three functional classes of polypeptides have been identified in TGEV virions: a nucleocapsid protein, a matrix protein and a peplomer protein forming the characteristic surface projections (Garwes et al., 1976). The peplomer protein E2, a highly glycosylated polypeptide of 200K to 220K, has been shown to elicit the production of neutralizing antibodies (Laude et al., 1986; Jimenez et al., 1986; Garwes et al., 1987) which are able to confer protection on suckling piglets (Garwes et al., 1978/79). At least four main antigenic sites have been defined on the E2 protein by means of topographical and functional mapping with monoclonal antibody probes (Delmas et al., 1986). Most of the neutralization-mediating determinants appeared to be grouped in two related sites, both conserved between virus strains. The majority of the epitopes critical in neutralization appeared to be sensitive to denaturation (Jimenez et al., 1986; Garwes et al., 1987). In addition, it is anticipated that the peplomer bears virulence-modulating determinants, as has been suggested in the case of MHV-JHM (Fleming et al., 1986).

Substantial information on peplomer functional organization has been accumulated for other coronaviruses. The spike protein of IBV comprises two or three copies each of two glycopeptides S1 (90K) and S2 (84K). S2 anchors the peplomer to the viral envelope through a short C-terminal hydrophobic domain and S1 is non-covalently attached to S2 (Cavanagh, 1983; Binns *et al.*, 1985). Neutralizing and haemagglutinating antibodies bind to the S1 subunit (Mockett *et al.*,

1984). Removal of S1 abolished infectivity but not attachment to cells (Cavanagh & Davis, 1986). Recently, comparison of the amino acid sequences of two IBV strains, Beaudette (Binns *et al.*, 1985) and M41 (Niesters *et al.*, 1986), led to the proposal that two candidates for neutralization epitopes are located near the S1 N terminus (Niesters *et al.*, 1986). In MHV, the 180K E2 protein is cleavable by host cell proteases or by trypsin to form two comigrating products of 90K. Palmitic acid has been found to be covalently attached to one of the 90K species, probably defining the membrane-anchored subunit. Proteolytic cleavage of E2 may be required for membrane fusion activities (Sturman *et al.*, 1985; Frana *et al.*, 1985). Three independent studies on MHV (Talbot & Buchmeier, 1985) and TGEV (Delmas *et al.*, 1986; Jimenez *et al.*, 1986) characterized an epitope resistant to antibody selection which might be essential for productive infection.

In this paper we present the complete sequence of the E2 gene of TGEV. The main features predicted from the primary structure of the encoded protein are described and compared with those previously reported for the IBV spike protein.

#### METHODS

cDNA cloning and sequencing. The strategy and protocol were as reported previously (Laude *et al.*, 1987). Briefly, purified genomic RNA was copied by reverse transcriptase using either oligo- $d(T)_{12-18}$  or a specific 30-mer primer (pE2: 5' CATCATCCTTAACAAAATTCTCTAGCAGAA). RNase T2-treated cDNA-RNA hybrids were dC-tailed and inserted in *Pst1*-cut dG-tailed pBR322. Transfection of *Escherichia coli* RR1 and selection of recombinant clones were performed following standard methods. 'Shotgun' DNA sequencing by Sanger's chain termination method and sequence analysis were accomplished as described previously (Laude *et al.*, 1987); part of the 6.47 clone was sequenced using a 15-mer oligonucleotide (p47) instead of the M13mp18 universal primer. Synthetic oligonucleotides were obtained by the beta amidite method using a Biosearch 8600 DNA synthesizer. DNA has been sequenced at least twice on each strand.

*N-terminal sequencing of protein E2.* Virion polypeptide E2 resolved by SDS-PAGE was purified by electroelution as described (Laude *et al.*, 1987), and about 100 pmol were analysed in a 'gas phase' Applied Biosystems 470A apparatus.

## RESULTS

The coordinates of the four sequenced cDNA clones on the restriction map of the genome are given in Fig. 1. (The pTG clones 6.3 and 6.47 were derived using pE2 as a primer.) In Northern blot analyses, the pTG2.26 insert was shown to contain sequences that hybridized only with the two largest RNA species detected in TGEV-infected cells: the genomic RNA and subgenomic RNA 2 (data not shown). DNA sequencing led to the identification of a 4341 base open reading frame (ORF), which was an obvious candidate for the E2 gene. The sequences encompassing the E2 ORF are presented in Fig. 2. A characteristic feature is that it is flanked by an identical sequence 5' ACTAAACTT 3' at each end. A homologous sequence has been identified within each intergenic junction (Rasschaert et al., 1987). The first consensus sequence is located 25 bases upstream from the potential ATG initiation codon, and maps at 8.25 kb from the 3' end of the genome, which is in agreement with the size estimates for RNA 2 (Hu et al., 1984; Jacobs et al., 1986; Rasschaert et al., 1987). The second consensus sequence is followed 22 bases downstream by an ORF putatively located at the 5' end of RNA 3 (partly shown in Fig. 2). The deduced sequence of the 1447 amino acid primary translation product and its hydrophilicity profile are shown in Fig. 2 and 3 respectively. A hydrophobic stretch with the characteristics of an eukaryotic signal peptide is predicted at the N terminus (Von Heijne, 1986). Indeed, partial N-terminal microsequencing demonstrated that the first 16 residues were absent from the virion-associated E2 protein, as its N-terminal sequence was found to be XXFPCSKLTXRTIGNQ. Accordingly, the mature product would be 1431 amino acids long. It has a predicted mol. wt. of 158316, comprising 126 acidic and 91 basic residues. There are 33.5%hydrophobic residues. Thirty-two sites for N-glycosylation (Asn-X-Ser or Asn-X-Thr) occur in the sequence, involving as many as 27.5% of the available Asn residues. Most of them are associated with a hydrophilic segment of the E2 polypeptide (Fig. 3).



Fig. 1. Restriction map of the region of TGEV genome encoding the E2 gene (bar). The positions of the four cDNA clones and of the two primers used are shown.

## DISCUSSION

cDNA copies of the TGEV genome covering the 5' coding region of mRNA 2 were sequenced. A single large translation frame was found, yielding a 1447 amino acid product with the characteristics of a coronavirus peplomer glycoprotein. The other identified ORFs did not exceed 200 bases and are within the E2 gene (not shown). The first ATG of the E2 ORF is positioned 24 bases downstream from a consensus sequence which is assumed to be the start of the mRNA 2 transcript (Fig. 2). The sequence upstream from the ATG codon (CACCATGA) is optimal for initiation by eukaryotic ribosomes [(CC)ACCATGG; Kozak, 1986]. Moreover the first Met is followed by a leader sequence that has been shown to be removed from the mature protein. The deduced cleavage site for signal peptidase is located after the 16th residue, between Gly and Asp. Inspection of the nucleotide data revealed the occurrence, 120 bases downstream, of an additional consensus sequence, TTCTAAACTA, which could function in the initiation of transcription. However, the next ATG in frame with the E2 ORF occurs only at position 520 and is not followed by a peptide sequence likely to translocate E2 into the membrane. Comparison of our results with previously reported partial nucleic acid data indicates a discrepancy at the 3' end of the E2 ORF, where the ORF is 3.9 kb long with GCCATGA at the 3' terminus (Hu et al., 1984). Our data prove that the sequence GCCTAGA occurs instead, at 3.8 kb from the initiation codon. Hence, the ORF extends up to a double stop sequence CCATTAAATTTAA occurring at 4.3 kb, and thereby includes the sequences predicting the anchor structure of the protein (see below).

The deduced mol. wt. of the virion-associated E2 is 158K (aproprotein), a value in close agreement with the  $M_r$  160K determined for the TGEV E2 unglycosylated form in tunicamycintreated cells (Jacobs *et al.*, 1986; B. Delmas & H. Laude, unpublished results). The 130K  $M_r$  species detected by translation of mRNA 2 in reticulocyte lysates might thus correspond to an incomplete translation product (Jacobs *et al.*, 1986). In the mature polypeptide, the carbohydrate moiety should approach 27% of the total weight, implying that a large proportion of the 32 potential sites for N-glycosylation are functional. An equivalent high sugar content has been reported for the IBV spike protein (Binns *et al.*, 1985).

The hydrophilicity profile predicts that E2 is hydrophobic overall (Fig. 3), reflecting the spatial importance of the tightly packed core in the peplomer. The amino half of the E2 chain shows few highly hydrophilic (virtually exposed) segments, whereas several prominent peaks are visible in the carboxy half. Examination of the sequence near the C terminus reveals the presence of a highly hydrophobic segment, comprising 45 unpolar residues, including 11 cysteines (Fig. 2). A similar structure has been previously noted in the IBV spike protein (44 hydrophobic residues including six Cys; Binns *et al.*, 1985). Such a high ratio of cysteine residues (24.5% as compared to 3.4% for the whole molecule) in the presumptive anchor region of the peplomer seems so far to be a distinctive feature of the coronaviruses. In both the viruses, the Cys residues cluster mainly in the carboxy distal half part of the hydrophobic domain. Hypothetically, these residues may serve as a site for covalent linkage of fatty acid chains (see Schmidt, 1983), as one E2 subunit of MHV has been reported to be acylated (Sturman *et al.*, 1985). Moreover, an eight residue segment, KWPWYVWL, probably corresponding to the site of entry into the membrane, is perfectly conserved in TGEV and IBV (Fig. 2). In both cases, this

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GACTATGTAATTAAABAAAAAAAAATTGAATGAAATGGTCATGGATTACTAABGAAGGGTAAGTTGCTCATTAGAAATAATGGTAAGTTACTAAAACTLTG GTAACACTTCGTTAACACCACC	
30 60 90 120   AT5RAAAAACTATTIGTGGTTTGTGGTGGTAATGGCAACCAGTGGGACAATTATGGAGCAACTATGGCAACCAGTGGGAATLEATTGGAACCCTICCTTGTTCCTAATTGACTAATAGAACTATAGGCAACCAGTGGGAATLEATTGAAACCTICCTTGTTCCTAATTGACTAATAGAACTAATAGAACCAGTGGGAATLEATTGAAACCTICCTTGTTCCTAATTGACTAATAGAACTAATAGAACCAGTGGGAATLEATTGAAACCTICCTTGTTCCTAATTGACTAATAGAACTAATAGCAACCAGTGGGAATLEATTGAAACCTICCTTGTTCCTAATTGACTAATAGAACCAATAGCAACCAGTGGGAATLEATTGAAACCTICCTTGTTCCTAATTGACTAATAGAACCAGTGGGAATLEATTGAAACCTICCTTGTTCCTAATTGACCAACTAATAGCAACCAGTGGGAATLEATTGAAACCTICCTTGTTCCTAATTGAACCAATTGCCAACCAGTGGGAATLEATTGAAACCATCCTTGTTCCTAATTGAACCAATTGAACTAATAGCAACCAGTGGGAATLEATTGAAACCAGTGCAACCAGTGGGAATLEATTGGAACCAATTGCCAACCAGTGGGAATLEATTGGAACCAACCAGTGGGAATLEATTGGAACCAATTGAATTGAAACTAATGGCAACCAGTGGGAATLEATTGGAACCAACCAGTGGAATTGCCAACTAGTGGCAACCAGTGGGAATLEATTGGAACCAATTGCCAACTAGTGGCAACCAGTGGGAATTGACCAATTGGCAACCAGTGGGAATTGACTAATGGAACCAATTGGCAACCAGTGGGAATLEATTGGAACCAATTGGAACCAATTGGAACCAATTGGAACCAATTGGAACCAATTGGCAACCAGTGGGAATTGACCAATTGGAACCAATTGGAACCAATTGGAACCAATTGGAACCAATTGGAACCAATTGGAACCAATTGGAACCAATTGGAACCAATTGGAACCAATTGGAACCAATTGGAACCAATTGGAACCAATTGGAACCAATTGGAACAATTGGAACCAATTGGAACCAATTGGAACAATTGGAACCAATTGGAACCAATTGGAACCAATTGGAACCAATTGGAACCAATTGGAACCAATTGGAACCAATTGGAACTGGAACAATTGGAACTGGAACTGGAATTGGAACTGGAATTGGAACTGGAATTGGAACTGAATTGGAACTGGAACTGAATTGGAACAATTGGAATTGGAACAATTGGAACAATTGGAACAATTGGAACAATTGGAACAATTGGAACAATTGGAACAATTGGAACAATTGGAACAATTGGAACAATTGGAACAATTGGAACAATTGGAACAATTGGAACAATTGGAATAGAATTGGAATTGGAACAATTGGAATT	40
150 180 210 240 CTARACCATASTAGTAGGTAGGTACCACCTAATTCAGATSATSTGTTAGGTGATGATGGTGATGATGGTAGGATGACTAGTAGGTAG	80
270 300 360 GAAAATCTTAAABCATTGTATTGGGATTATGCTACAGAAAATATCACTTGGAATCACCGGAGCGACGGTTAAAGCATGTCTTATAGGATACCCGTACCGGTTACAACAAGCCGG ENLKALYWDYATE <u>NI</u> WNHRDRLNVVVNGYPYSITVTI R	120
480 480 AATITTAATTCTGCTGAABGTGCTATTATATGCATTISTAAGGGGCTCACCACCTACTACCACCACAGAATCTAGTTGGAGTTGGGGTAGTGGGTGG	160
510 CCTATATGTCCTTCTAATTCAGAGGCAAATTGTGGTAATAGGCTGTATGGCCGTACAATGGCGTTGCCGTAGTGACGTGGTGCGGTAGTGGCGTAGTGGCGTAGTGGCGTAGTGGCGTAGTGGCGGTAGTGGCGGTAGTGGCGGTGGCGGTGGCGGGGGGGG	200
630 660 720 CAATGGTCTGGCACTGTCACATTGGTGGTATGCCGGCGACACATTAGAAGCCGTGGCACCTTGTGGGGTATAATCCGGTTATGGTGGTAGTGTAATGCGGTATTATAGGGTTAAT DNSGTVTFGDMRATTLEVAGTLVDLNNFFNPVYDVSYYRVN	240
750 780 880 840 AATAAAAATGGTACTACCGTAGTTICCAATGGCAGGTGGTGATGTGCTAGTGTTITAGTACTACACAGCCAGGAGGTTITATACCATCAGATTITAGTTITAGTATAGTGG N K <u>N G T</u> T V V S <u>N C T</u> D Q C A S Y V A N V F T T Q F G G F I P S D F S F N N W	280
B70   900   930   960   9	320
990 GABGGTGCTGGCTTIGATCAATGGTAGTGGTGCTGTATAGGTGCAATGGTGCAATGGTGCCAAGATGAGGGTGCCAAGGTGCCAAGATGAGGGTGCCAAGGGTGCCAAGGTGTGCCAAGGGTGCCAAGGGTGCCAAGGGTGCCAAGGTGTCAAGGTGCCAAGGTGCCAAGGGTGCCAAGGGTGCCAAGGGTGCCAAGGGTGCCAAGGGTGCCAAGGGTGCCAAGGTGCAAGGGTGCAAGGGTGCAAGGGTGCAAGGTGCAAGGTGCAAGGGTGCAAGGGTGCAAGGGTGAAGGTGCAAGGGTGCAAGGGTGCAAGGGTGCAAGGGTGAAGGGTGAAGGGTGAAGGGTGAAGGGTGAAGGGTGAAGGGTGAAGGGTGAAGGGTGAAGGGTGAAGGTGAAGGTGAAGGTGAAGGTGAAGGGTGAAGGTGAAGGTGAAGGTGAAGGGTGAAGGTGAAGGGTGAAGGTGAAGGTGAAGGTGAAGGTGAAGGTGAAGGTGAAGGGTGAAGGGTGAAGGGTGAAGGGTGAAGGGTGAAGGGTGAAGGGTGAAGGGTGAAGGGTGAAGGGTGAAGGGTGAAGGGTGAAGGGTGAAGGGTGAAGGTGAAGGTGAAGGTGAAGGTGAAGGTGAAGGTGAAGGTGAAGGTGAAGGTGAAGGTGAAGGTGAAGGTGAAGGTGAAGGTGAAGGTGGT	360
1110 1140 1140 1170	400
1230 1260 1290 1320   CACTATAATGBCACAGCTCTTAAGTATTAGGAACATTACCACCTAGTGTCAGGAGGAGATTGCTATTAGGAGGGGGGCCATTITATATTAATGGTTACGATTICTTAGCACATTICCT 1320   H Y N G T A L K Y L G T L P P S V K E I A I S K W G H F Y I N G Y N F F S T F P	440
1350 ATTGATTGTATATCTITTAATTTGACCACTGBTGATAGTGACGATTTGCGGACAATAGCTTACCACGCGCACCACGCAGCACACGAGGTGAAAACACGACGCTATTACAAAGGTGACG I D C I S F N L T T G D S D V F W T I A Y T S Y T E A L V Q V E N T A I T K V T	480
1470 TATTETAATAGTCACETTAATAGACATTAAATECTICCAAATTACTGCCAATTAGAATTATCGGATTITATCCTGTTACTGCAAGTGGAGTTGGCCTTGTCAATAAGAGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTG	520
1620 CCTAGCTTTTACCATAGCTATGTTAACTATGGTCTTGGTATGAASCGTAGGGGTATGGGTCAACCCATAGCCTCAACATTAAGTAACTGACGACTGACGATGCAGGATGAC PSFYTHTLVNLT GLGMKKSGYGQPLASTLSNLTLLPMQDM	560
1710 1200 AACACCEAATBTGTACTGTATTGGTTATGTTCATTTCAGTTATGTTGAAAABJGCTTTATBBGACAATATTTTTAABCGAAACTGCACGGACBTTTTGAGTGCACACA N T D V Y C I R S D Q F S V Y V H S T C K S A L W D N I F K R N C T D V L D A T	600
1830 1840 BECTETTATAAAAACTEGETACTITECTTICTCATTITEAAATTEGAAATTEGAAATTEAACATTETTAACAAAETTCTGTTETCETTEGEGECTAATTETAAGTTEGAAATTEGAAGTTEGAATTEGAAATTEGAAATTEGAACAATTAACAAAETTCTAACATTETTAACAAETTCTGTTEGAEGCTGATTEGAEGCTAATTETAAATTEGAAATTEGAAATTEGAAATTEGAAATTEGAACAATTAACAAETTCTTAACAAETTCTGTTEGAETTGGAEGCTGATTEGAAATTEGAAATTEGAAATTEGAAATTEGAACAATTAACAAETTCTTAACAAETTCTGTTEGAETTGGAEGCTGATTEGAAATTEGAAATTEGAAATTEGAACAATTAACAAETTCTTAACAAETTCTGTTEGAETTGGAEGTCTGTTEGGEGCTAATTEGAAATTEGAAATTEGAAATTEGAACAATTAACAAETTCTTAACAAETTCTGTTEGAEGTCTGTTEGGEGCTAATTEGAAGTTEGATEGAEGTT A V I K T G T C P F S F D K L N N Y L T F N K F C L S L S P V G A N C K F D V A	640
2010 . 2040 GCCCGTACAABAACCAATGABGATGTTAGTAGATIGTAGTAATATATAGTAGAGAACAAACATAGTGGGTGTACCGTCTGATAATAGTGGGTGTGCACGATTGTCAGTGCTACAC A R T R T N E G V V R S L Y V I Y E E G D N I V G V P S D N S G V H D L S V L H	680
2070 2160 CTAGATTCTGCACAGATTACAATATATATGGTAGAACTGGTGTGTGGTGTGGTGTGTGT	720
2190 2220 2250 2280 TITAAAAAATGITAGIGATGGIGATGGIGATGGIGACGCCATGGIGAGGGGCGGIGATGGIGGGGCCATCACTGCCATGACGGGGACCGITA F K <u>N V S</u> D G V I Y S V T P C D V S A Q A A V I D G T I V G A I T S I N S E L L	760
2310 2340 2340 2370 2370 2370 2400 2370 2370 2400 2370 2370 2400 2570 2570 2400 2570 2570 2570 2400 2570 2570 2400 2570 2570 2570 2570 2400 2570 2570 2570 2570 2570 2570 2570 25	800

. , 2430 , .	2460	2490 2520	
ACCTATICIAACATAG61G111GTAAAAAT6G1GCT1T1G11T1TATTAACG1C	ACACATTCTGATGGAGACGTGCAACCAATT	AGCACIGGTAATGICACGATACCIACAAACITIACC	
TYSNIGVCKNGAFVFI <u>NV</u>	THSDGDVQPI	STG <u>NVT</u> IPT <u>NFT</u>	840
355 Ú	2504	2/12	
2300	2380	2010 . , 2040 ACCIGIAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG	
			880
			000
2670	2700	2730	
16TCAAACTATTGAGCAAGCACTTGCAATGGGTGCCAGACTTGAAAACATGGAG	GTIGATICCATGTIGTITGTTTCIGAAAAT	GCCCTTARATTGECATCTGTTGAAGCATTCAATAGT	
COTIEOALAMGARLENME	VDSNLFVSEN	ALKLASVEAFNS	920
	2820	2850	
TCAGARACTITAGACCCTATTTACAAAGAATGGCCTAATATAGGTGGTTCTTGG	CTABAABGTCTAAAATACATACTTCCBTCC	CATARTAGCARACGTARGTATCETTCABCTATAGAR	
SETLDPIYKEWPNIGGSW	LEGLKYILPS	HNSKRKY RSAIE	960
~			
	2940	2970	
GACTIGCTITITGATAAGGTTSTAACATCIGGTTAGGTACAGTTGATGAAGAT	TATAAACGTTGTACAGGT6GTTATGACATA	GCTGACTTAGTATGTGCTCAATACTATAATGGCATC	
DLLFDKVVTS6L6TVDED	YPRCTSGYSI	ADLVCARYYNGI	1,000
7070	70/0	7000 7400	
	3060	3090	
		C C C C A U A T D C ATU A	1 0/0
		3 G G H V H I F F H V H	1.040
3150	3180	3240	
STICAGECTAGACTTAATTATGITSCIC CACABACTGATGTATTGACABACAG	CASCAGATICIGECIAGIGCIIICAATCAA	GCTATIGGIAGCATIACACAGICATITGGIAAGBTT	
VIGARIUNYVALDTOVINIKN	DUILASAFNO	ALSINITOSFOKV	1,080
3270 , .	3300	3330 3360	
AATGATGCTATACATCAAACATCACGAGGTCTTGCTACTGTTGCTAAAGCATTG	SCAAAAGTGCAAGATGTTGTCAACATACAA	GGGCAAGCTTTAAGCCACCTAACAGTACAATTGCAA	
N D A I H D T S R G L A T V A K A L	A * V & D V V N I &	GOALSHLTVQLQ	1.120
	3420	3450 3480	
AATAATTICCAAGCCATTAGTAGTICTATTAGTGACATTTATAATAGGCTTGAC	SAATTGAGTGCTGAT <u>GCACAAGTTGACAG</u>	CTEATCACAGGAAGACTTACASCACTTAATSCATTT	
NNFQAISSISDIYNRLD	ELSADAÐVDR	LITGRLTALNAF	1.160
	<u> </u>		
	354u	3570	
BISTUTCABACTUTARLCAGACAGAGGGGGGGTTAGGGGTAGTAGGACAACTTGCU	AAAGPCAAGGI FAA*GAA ISCGI TAGGTC F	CAGTETCAGAGATICGGATICIGIGGIAATGGTACA	
V S V I L I K U K E V K K S K S L A	1 VINEL 9 8 3	USJAFSFLUNGI	1,200
3-30	14	3. P., 717.	
F617161111F66T06Pa6616F66P66464766C4111111111	STEFIATEACCAACECTTATEAAACTETE	1010	
HIESLANAAPNGINIEEHT		TAMPRICASD6	1.240
			11240
	3780	3810	
CGCACTTTTGGACTTGTCGTTAAAGATGTCCAGTTGACTTTGTTTCGTAATCTA	GATGACAAGTTCTATTTGACCCCCAGAACT	ATGTATCAGCCTAGAGTTGCAACTAGTTCTGACTTT	
RTFGLVVKDVBLTLFRNL	DDFFYLTPRT	MY Q P R V A T S S D F	1.280
	3900	3930 396ú	
GTTCAAATIGAAGGGTGCGATGTGCTGTTTGTTAATGCAACTGTAAGTGATTTG	CCTAGTATTATACCTGATTATATIGATATT	AATCAGACIGTTCAAGACATATTAGAAAATTITAGA	
VQIEGCDVLFV <u>NAT</u> VSD <u>1</u>	PSIIPOYIDI	<u>NQT</u> VQD1LENFR	1.320
774			
	40_0 .	4080	
	AALLIDAL: SEISAAAAIIGAIGALIIAGAA	ITTRUS CAGARAASL ACATARCACCACTGTAGAA	4 260
	<u></u>	FRSEFLANISVE	1.300
4110	4140	4170 4200	
CITECCATTCTCATTERACAGEACACTACATCATCATCTCEATCTC	1140 . TATRATAGAAAT (TATRATAGAAT (TAGRATAGAATAGAATAGAATAGAATAGAATAGAATAGA	TRETATRIESCIACIAATARECTIACIACIAATA	
			1 400
			1.400
4230	4260 .	4290	
TITTECATACCATTACTECTATTTECTETTETAGTACAGETTECTETSGATEC	ATAGGTIGTTTAGGAAGTIGTIGTCACTCT	ATATGTAGTAGAAGACAATITGAAAAATTACGAACCA	
F 🖸 I P L L F 🛈 🛈 S T S 🛈 🛈 G Ć	1 6 🛈 L 6 S 🛈 🛈 H S	t 🛈 S R R Ø F E N Y E P	1.440
4350	4380	4410	
HILDHARAAALIGCACGICCALLARATITAAAATGTTAATTCTATCATCTGCTAT	ARTRECASTIGITICISCIAGAGAATITIS	ITTAAUUATUATUAATAAAGTCTTTAAGAACTAAACT	
4470	4540	45.70	
TACGARTEATTACAGARTETGTATAGARAGATATTACAGARTEAGARTEATTACAGARTEAGARTEATTACAGARTE	ANN	10100	
ΝΟΥΚΟΥΤΟΙ		Y F A V T	

Fig. 2. The nucleotide sequence and the predicted amino acid sequence of the peplomer protein of the Purdue-115 strain of TGEV. The consensus decanucleotides (see text) are boxed. Proximal ATG codons are underlined, stop codons are overlined. Amino acids are numbered at the right. Potential sites for N-glycosylation (NXT or NXS) are underlined. The potential signal peptide and membraneanchoring domain are indicated by open and closed lines respectively. Homology regions (at least three consecutive matches) with the spike sequence of the Beaudette strain of IBV are boxed.



Fig. 3. Hydrophilicity plot of the TGEV E2 precursor polypeptide. Running average taken over an hexapeptide using the hydrophilicity values of Hopp & Woods (1981). Bars in the upper panels indicate the *N*-glycosylation sites; hatched areas represent the signal peptide and the anchoring domain respectively; dotted area indicates a predicted long amphipathic  $\alpha$ -helix; the relative positions of the IBV spike protein signal ( $\bigtriangledown$ ) and connecting ( $\blacktriangledown$ ) peptides are indicated.

	а	b	С	d	e	-f	g
Hydrophobic residues	5/7	7/7	3/7	3/7	6/7	2/7	0/7
1063	-L	А	S	А	F	Ν	Q
	А	Ι	G	Ν	Ι	Т	Q
	S	F	G	К	V	Ν	D
	А	Ι	Η	Q	Т	S	R
	G	L	А	Τ	V	А	К
	А	L	А	Q	V	Q	D
	V	V	Ν	Ì	Q	G	Q-111

Fig. 4. Search for a stable elongated structure in the TGEV peplomer. The amino acids are listed horizontally following a heptad pattern (two  $\alpha$ -helix turns). Residues in the columns b and e may form the interface between the chains in a  $\alpha$ -helical coiled-coil structure.

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region of E2 is preceded by a cluster of N-glycosylation sites, starting at a markedly hydrophilic stretch of 20 amino acids (Fig. 3). The C-terminal hydrophilic segment of TGEV E2 (16 amino acids) is significantly shorter than that reported for both the Beaudette and M41 strains of IBV (Binns *et al.*, 1985; Niesters *et al.*, 1986). Recently de Groot *et al.* (1987) have described the presence of two heptad repeats in the peplomer proteins of MHV, IBV and feline infectious peritonitis virus, which are indicative of a coiled-coil structure. This structure could provide an explanation for the elongated shape of the peplomer. A Fourier transform of the distribution of hydrophobic residues in the TGEV E2 chain allowed us to characterize a segment of about 55 residues having a strong propensity to form an amphipathic structure with dominant periodicity of 100°  $\pm$  20° (De Lisi & Berzofsky, 1985). This segment is located in a region of E2 which is devoid of both Pro and Cys residues (1037 to 1184). In addition, few aromatic residues are present in the heptapeptide repeat (Fig. 4). This predicts an 8 nm long  $\alpha$ -helical, possibly coiled-coil segment.

Three other features were noted while aligning optimally the TGEV and 1BV E2 protein sequences (not shown). First, the overall highest homology obtained by Dayhoff's alignment is  $32\cdot3\%$  (with  $12\cdot5\%$  residues unmatched) which is consistent with the fact that the two viruses belong to separate antigenic groups. Most of the stringent homology regions (boxed in Fig. 2) cluster in the carboxy halves of the molecules. In particular, a hydrophilic stretch of 11 residues at position 1144 (TGEV precursor) is perfectly conserved. The sequences are markedly divergent in the amino part, except for one conserved region at positions 686 to 697. Second, a

basic sequence DRTRG occurs in TGEV E2 at position 782, at about the same distance from the C terminus as the sequence RRFRR in the IBV spike protein, where the S1/S2 cleavage site has been demonstrated (Cavanagh *et al.*, 1986). Third, the predicted TGEV E2 mature protein contains 287 residues more than the IBV protein, a difference expected from the comparison of their respective  $M_r$  values. The characteristic Lys-Val-Thr twofold repeat present in the IBV signal peptide is conserved in the TGEV E2 homologous sequence (positions 289 to 296). This, along with a tentative alignment of the sequences, suggests that the extra TGEV E2 sequence largely protrudes at the NH<sub>2</sub> terminus. Whether or not a specific function is associated with this sequence is not clear.

This paper, together with two papers reporting the sequences of the nucleocapsid N (Kapke & Brian, 1986) and the transmembrane E1 proteins (Laude *et al.*, 1987), provides a complete set of data on the major structural proteins of TGEV. The availability of cloned TGEV peplomer sequences, along with a panel of monoclonal antibodies and of neutralization-resistant mutants will allow localization of functionally important epitopes.

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