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Processing and Antigenicity of Entire and Anchor-Free Spike Glycoprotein S of Coronavirus TGEV Expressed by Recombinant Baculovirus

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The S gene of transmissible gastroenteritis virus (TGEV) was inserted into the genome of *Autographa californica* nuclear polyhedrosis virus (AcNPV) using the transfer plasmid pVL941. Infection of Sf9 insect cells with the recombinant virus resulted in the synthesis of a 175K polypeptide which was able to trimerize and was transported to the cell surface as is the authentic TGEV S protein. Despite the lack of complete carbohydrate processing, the recombinant S protein exhibited antigenic properties similar to TGEV S and induced high levels of neutralizing antibodies in immunized rats. Engineering a deletion (70 amino acids) into the carboxy-terminus containing the membrane anchor of the polypeptide allowed its secretion. The oligomerization process and the antigenic profile of the anchor-free S protein were shown to be partially altered.

INTRODUCTION

Transmissible gastroenteritis virus (TGEV) causes an acute enteritis which is often fatal in newborn pigs. It belongs to the *coronaviridae,* a family of enveloped positive-stranded RNA viruses. Virions are composed of a nucleocapsid protein and 2 or 3 envelope glycoproteins. The spike, or peplomer protein S (formerly named E2), forms large surface projections characteristic of coronaviruses. It exhibits a typical class I glycoprotein profile with a cleaved N-terminal sequence and a transmembrane segment located near the C-terminus. Studies on murine coronavirus MHV have implied a role for S protein in membrane fusion and in attachment of the virus to host cell receptor (for a review, see Spaan *et al.,* 1988).

Nucleotide and amino-terminal sequence data have shown that the mature TGEV S polypeptide contains 1431 (Purdue-115 strain) or 1433 (Miller and FS772/70 strains) amino acids (Rasschaert and Laude, 1987; Wesley, 1990; Britton and Page, 1990). In addition, S protein of porcine respiratory coronavirus PRCV (a nonenteropathogenic virus closely related to TGEV) has been shown to lack 224 residues at the N-terminus (Rasschaert *et al.,* 1990). Recent studies on TGEV have led to the proposal that the coronavirus spike is a homotrimer (Delmas and Laude, 1990). The S oligomer is possibly stabilized by an interchain coiled-coil elongated structure predicted from the sequence, near the C-terminal quarter of the polypeptide chain (De Groot *et al.,* 1987; Rasschaert and Laude, 1987).

The S protein of TGEV is the only antigen to elicit the production of highly neutralizing antibodies (Laude *et al.,* 1986; Jimenez *et al.,* 1986) assumed to mediate the passive protection conferred to suckling piglets (Garwes *et al.,* 1978/79). The antigenic structure of S protein has been studied using monoclonal antibodies. At least five major groups of epitopes or sites have been identified (Delmas *et al.,* 1986; Correa *et al.,* 1988), and all are located in the amino-half part of the ectodomain (Correa *et al.,* 1990; Delmas *et al.,* 1990; Posthumus *et al.,* 1990). Most of the epitopes critical for neutralization have been found to be conformation-dependent (Correa *et al.,* 1988). Cotranslational glycosylation has been shown to be an essential requirement for both expression and maintenance of most of the major antigenic sites (Delmas and Laude, 1991).

The baculovirus *Autographa californica* (AcNPV) has been widely used as a helper-independent vector for high level expression of foreign genes (Luckow and Summers, 1989). We have attempted to express the TGEV S gene in this vector in order to synthesize enough protein for eventual structural and functional analyses. In this paper, we report the isolation of a recombinant baculovirus expressing the complete S coding sequence. Infection of insect cells with this virus resulted in the production of S protein with properties similar to the authentic S. We also report the engineering and characterization of an S protein lacking the membrane anchor, which is secreted from infected cells.

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MATERIALS AND METHODS

Cells, viruses, and monoclonal antibodies

AcNPV and recombinant baculoviruses were isolated, grown, and assayed in confluent monolayers of *Spodoptera frugiperda* cells (clone Sf9) maintained in Hink's medium supplemented with 10% fetal calf serum according to standard procedures (Summers and Smith, 1987). Propagation of Purdue-115 strain of TGEV in the pig cell line PD5, preparation of radiolabeled virus, and neutralization assays were done as previously described (Laude *et al.,* 1986). The characteristics of monoclonal antibodies (mAbs) in terms of polypeptide specificity, neutralizing ability and epitope mapping have been published elsewhere (Laude *et al.,* 1986; Delmas *et al.,* 1986). Mab 3b5, which recognized a linear, glycosylation-independent epitope (Delmas *et al.,* 1990) was included in all experiments.

Construction of the TGEV S gene from cDNA

A full-length cDNA copy of the TGEV S gene coding sequence was constructed using the plasmids pTG6-47, pTG6-3, pTG6-5, and pTG6-39 (Rasschaert *et al.,* 1987) following the general scheme outlined in Fig. 1. Briefly, the *Hpa*I–*Bst*EII fragment derived from pTG6-47 and the *Bst*EII–*Xho*I fragment derived from pTG6-3 were sequencially inserted into the *Sma*I–*Pst*I cloning site of pTZ18R, resulting in the plasmid pZG11. The complete S gene was obtained by a three-way ligation of the *Xho*I–*Kpn*I and *Kpn*I–*Pst*I fragments excised from pTG6-5 and pTG6-39, respectively, into *Xho*I–*Pst*I digested pZG11. The resulting plasmid, named pZG35, contained a 4.5-kbp insert. The sequence of the insert was verified by supercoiled DNA sequencing (Lim and Pène, 1988) using specific oligonucleotides. It diverged from the reported sequence (Rasschaert and Laude, 1987) by only three silent nucleotide changes, located within the sequence taken from pTG6-39.

Construction of transfer vectors pVL941S and pVL941St and generation of recombinant baculoviruses

A *Sst*I–*Pst*I fragment (4460 bp) excised from pZG35 was subcloned into the *Bam*HI site of the baculovirus transfer vector pVL941 (Luckow and Summers, 1989), using unphosphorylated 8-mer linkers (5'GATCAGCT; 5'GATCTGCA) and T4 DNA ligase. The resulting plasmid was designated pVL941S. A second plasmid, pVL941St, was constructed by inserting a truncated S gene. The insert was produced by PCR mutagenesis (Scharf *et al.,* 1986) on pZG35 using the oligonucleotides 5'AATTCGAGCTCGGATCCCAACACACCA and 5'TCAATTCTATGGATCCATTCAAGATTG as 5' and 3' amplimers, respectively. The amplification product was digested by *Bam*HI and ligated into the pVL941 cloning site. The orientation and sequence of the S and St inserts relative to the AcNPV polyhedrin leader were determined by restriction analysis and partial DNA sequencing. Transfer of the TGEV S and St genes into the AcNPV genome was accomplished by transfection of Sf9 cells as described by Summers and Smith (1987). Recombinant baculoviruses, designated Ac941S and Ac941St, were screened by dot blot hybridization using a TGEV S-specific, [^{32}P]labeled DNA fragment as a probe. Polyhedron-negative clones were identified and amplified.

Radiolabeling and immunoprecipitation

Monolayers of Sf9 cells were infected with Ac941S or Ac941St at a multiplicity of 10 PFU per cell and incubated at 28°C for 24 hr. The medium was then replaced with methionine-free Grace medium containing 5% fetal bovine serum and 100 μCi of [^{35}S]methionine (600 Ci/mmol, Amersham) per ml. In experiments including a chase period, cells were rinsed and incubated for additional periods with complete medium. Cell lysates were prepared in RIPA buffer, immunoprecipitated with mAb 3b5 and analyzed by SDS–PAGE in reducing conditions as described (Laude *et al.,* 1986).

Endoglycosidase digestion

These experiments were performed as described by Domingo and Trowbridge (1988). Immune complexes were eluted from protein A–Sepharose by boiling 3 min in 1% 2-mercaptoethanol + 0.2% SDS. The mixture was 15-fold diluted with 50 m*M* sodium phosphate, pH 6.0 + 0.5% Nonidet P40 and 0.1 *M* 2-mercaptoethanol and incubated with 100 mU/ml of endoglycosidase H (Endo-H; Boehringer) for 2 hr at 37°C.

Rate zonal centrifugation and cross-linking experiments

These procedures have been described elsewhere (Delmas and Laude, 1990). Briefly, aliquots of labeled infected cell extracts were applied to a linear 5–20% sucrose gradient in RIPA buffer and ultracentrifuged at 5°C. Aliquots were immunoprecipitated directly or after cross-linking with dimethylsuberimidate (DMS; Merck). Crosslinked samples were analyzed by electrophoresis on a 3.5% polyacrylamide gel run in 0.1 *M* Tris–borate (pH 8.5). Crosslinked phosphorylase b (Sigma) served as a size marker.

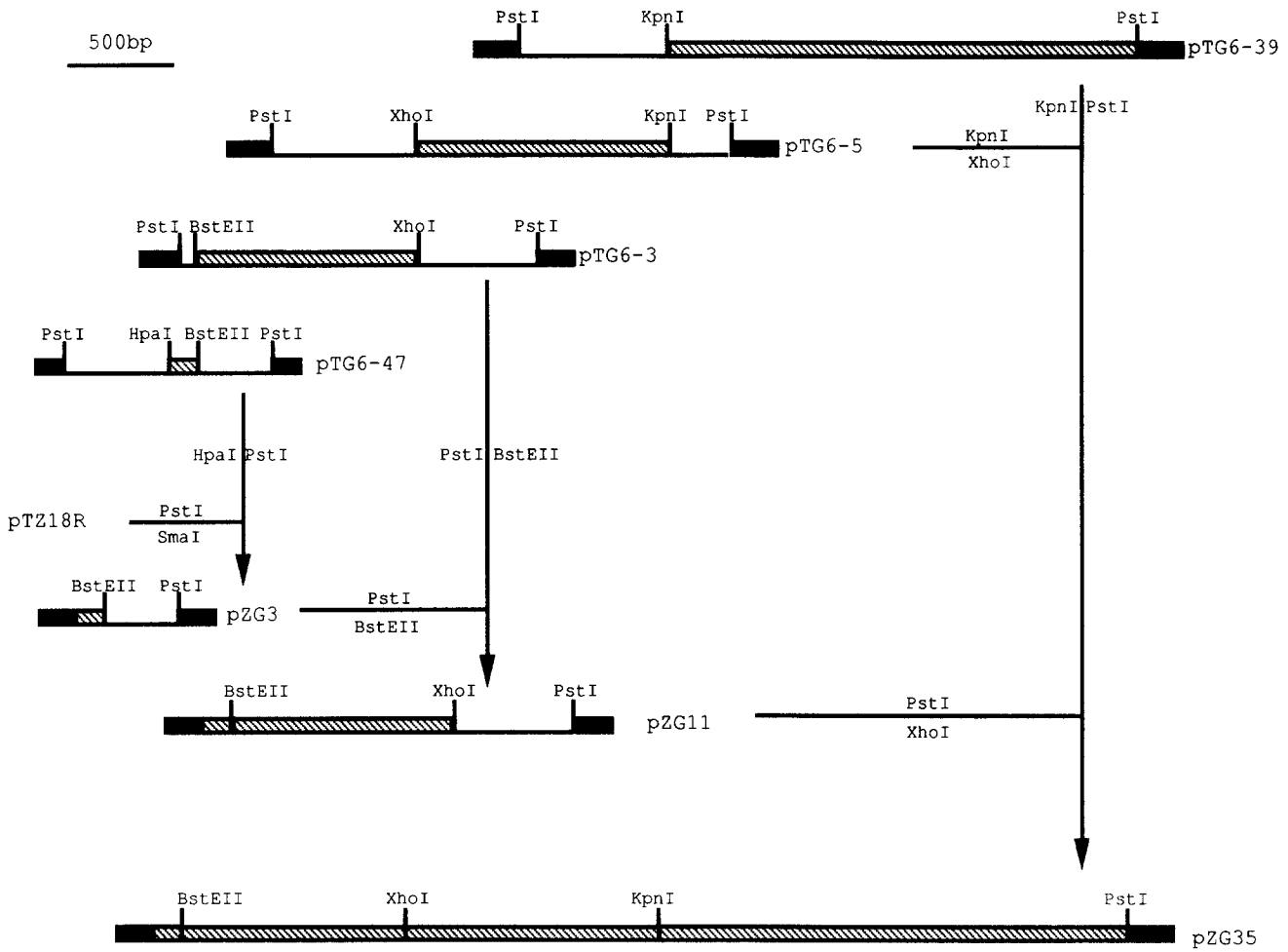

FIG. 1. Schematic diagram of the construction of the TGEV S gene from the Purdue cDNA. Hatched bars represent cDNA fragments used for the reconstruction of the complete coding sequence of the S gene. Additional details about the construction are given under Methods.

Immunofluorescence assays

Suspension cultures of *S. frugiperda* cells were infected with baculoviruses at a multiplicity of 10 PFU per cell. At 48 hr p.i., cell aliquots were removed and spotted onto glass slides. Acetone-fixed cells were stained using mAb 3b5 and FITC rabbit anti-mouse IgG (Biosis). Living cells were stained by incubation for 1 hr at 4°C with ascites fluids 100-fold diluted in Grace medium then with FITC conjugate.

Immunization experiments

Ac941S-infected cells (m.o.i. 10 PFU) were collected at 70 hr p.i., washed, and resuspended in PBS. The cells were frozen, thawed, and emulsified in Freund's complete adjuvant (v/v). Three 5-week-old female Lewis rats were primed via the intradermal or intraperitoneal route with the equivalent of 3×10^7 cells. The animals were boosted with a cell suspension without adjuvant. Serum samples were collected 12 days after the last injections and tested for neutralizing activity.

RESULTS

Construction of recombinant baculoviruses encoding entire or truncated TGEV S protein

Two plasmids were derived from baculovirus transfer vector pVL941: (i) pVL941S, which contained a full-length cDNA copy of TGEV S gene, was constructed as described under Methods. In this construct, the 5′ and 3′ extremities of the S coding sequence were positioned at 26 and 100 bp from the *Bam*HI cloning site, respectively. (ii) pVL941St contained a truncated version of S gene which lacked the 3′-last 210-nt sequence encoding the transmembrane and cytoplasmic domains. The corresponding insert was obtained by PCR mutagenesis. The amplimers were chosen to introduce a *Bam*HI site at each end, thus allowing direct insertion into pVL941 cloning site.

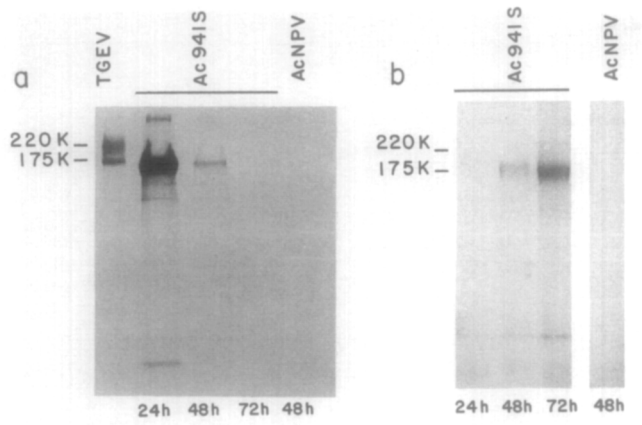

FIG. 2. Time-course of synthesis and accumulation of rS protein in insect cells infected by recombinant baculovirus Ac941S. (a) Ac941S- or AcNPV-infected Sf9 cells were labeled with [^{35}S]methionine for 4 hr at the indicated time p.i. Extracts from 3×10^4 cells were immunoprecipitated using anti-TGEV mAb 3b5. The proteins were resolved by SDS–PAGE on a 8.75% gel and visualized by fluorography. A sample from TGEV-infected PD5 cells was processed in a similar way for comparison. The species 175K represents an intracellular precursor of S, named S′ (see text). (b) Extracts from 4×10^6 cells harvested at the indicated time postinfection were processed as above. Immunoprecipitated material was visualized by staining with Coomassie blue.

In the resulting construct, the initiator codon of St sequence was located 9 bp downstream from the *Bam*HI site. At the 3′ end, St-specific sequence was fused with 25 codons provided by the vector sequence. Therefore, the 70 C-terminal amino acids downstream from Trp at position 1361 in the mature S protein were replaced by the following sequence: ILSWDPARTKNSLSSRKSVMLNPTR.

Expression of TGEV S polypeptides in insect cells

The expression of recombinant TGEV S polypeptides (rS) in Sf9 cells was monitored by immunoprecipitation of cell lysates or culture medium from labeled Ac941S-infected cells, followed by SDS–PAGE electrophoresis. A high Mr major protein species was synthesized (maximum at 24 hr p.i.) by Ac941S-infected cells (Fig. 2a). Its Mr was notably lower than the 220K mature species produced in TGEV-infected cells. This rS protein comigrated with the cotranslationally glycosylated intracellular 175K species S′ described by Laude *et al.* (1986). The time course for the accumulation of rS was found to be maximal at 72 hr postinfection (Fig. 2b). The yield of rS produced in insect cell cultures was estimated to be 2 μg/10^6 cells when compared to known concentrations of myosin following staining with Coomassie blue. The amount of rSt product was about twofold higher as compared to its untruncated homologue (data not shown).

Intracellular localization of rS protein

Ac941S-infected Sf9 cells were examined by indirect immunofluorescence at different times postinfection (Fig. 3). In fixed cells, the S gene product was primarily distributed in a perinuclear area, suggesting an accumulation of the antigen in the endoplasmic reticulum. In living cultures examined at 48 hr, a surface fluorescence was visible in certain cells in the population, which was indicative of antigen expression at the outer surface of the plasma membrane. No surface fluorescence could be detected at 24 hr p.i.

Oligomerization state of rS and rSt proteins

Gradient centrifugation and crosslinking experiments were performed in order to determine whether baculovirus-expressed S proteins existed in a trimeric form as found with the TGE virion-associated S protein. Results are presented in Figs. 4 and 5. In contrast to the authentic S protein, rS material exhibited a nonspecific aggregation as indicated by spreading of the protein from fraction 7 to the bottom of the gradient (Fig. 4a). Nevertheless, a peak of rS protein was clearly visible in fractions 13–15 (trimeric range of the gradient). Higher Mr species present in the same fractions were interpreted as nondissociated oligomers. A typical crosslinking pattern showing the presence of three main bands with respective Mr 175, 350, and 530K was observed with material obtained from fractions 13–15 (Fig. 4b). When the same experiments were performed with rSt protein, no evidence of a trimeric form was found (Figs. 5a and 5b).

Glycosylation state of rS and rSt proteins

Pulse-chase experiments were performed in order to analyze the processing of rS and rSt proteins in insect cells (Fig. 6). The resulting lysates were immunoprecipitated and treated or not with endo-H prior to the SDS–PAGE analysis. Pulse-labeled rS and rSt proteins exhibited a slightly larger Mr (around 5–10K) as compared to their 24-hr chased homologues (Fig. 6). In addition, both rS and rSt unchased proteins were found to be completely endo-H sensitive as revealed by a Mr shift to 160K, a Mr value similar to that of the apoprotein (Delmas and Laude, 1991). In contrast, endo-H treatment of 24-hr chased material revealed the presence of a species of intermediate Mr (about 165K), interpreted as being partially resistant to the enzyme. The carbohydrate processing occurred at a very slow rate since unprocessed molecules were still observed after a 24-hr chase period. As a consequence, S protein synthesized in Sf9 cells is detected as a double band—comprised of endo-H resistant and sensi-

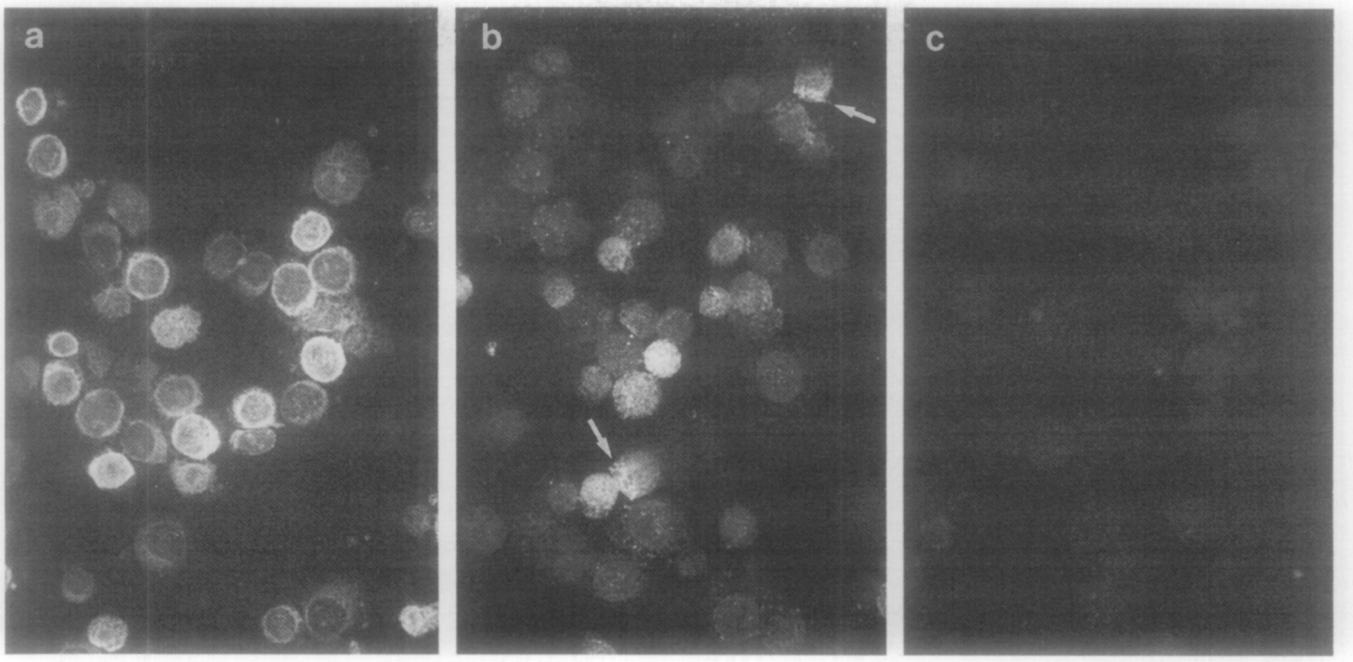

FIG. 3. Immunofluorescence assay of Sf9 cells expressing recombinant rS protein. Cells were infected with Ac941S (a, b) or AcNPV (c). Two days after infection, cells were incubated with anti-S mAb 3b5 then FITC conjugate with (a) or without (b, c) prior fixation. Arrows point to cells showing evidence of antigen capping, strongly suggestive of membrane surface fluorescence.

tive molecules—when a long-lasting labeling is performed (see Figs. 4a and 5a). It was also noted that the proportion of endo-H resistant molecules was greater for rS than for rSt protein.

Secretion of truncated rSt protein

Pulse-chase analysis was used to determine whether deletion of the anchor region would result in the secretion of S protein into the culture medium. Results shown in Fig. 7 indicate that a large proportion of rSt accumulated in the extracellular fraction of infected cells, whereas rS protein remained essentially cell-associated. Moreover, the extracellular rSt consisted of the lower Mr species previously shown to be endo-H resistant.

Antigenic analysis of S and rSt proteins

The antigenic properties of recombinant S and St proteins were evaluated using a panel of 22 TGEV S-specific mAbs (Fig. 8). We concluded that: (i) the reactivity of rS proteins toward the whole panel was similar to that of the authentic S protein (no variation exceeded 1 log5 between the two proteins. (ii) Significant discrepancies of reactivity, however, were noted between rS and rSt proteins, i.e., the latter exhibited a weaker reactivity ($\geq$2 log5) toward the majority of mAbs directed to A and B sites. Immunoprecipitation analyses comparing the two protein species fully confirmed this observation (data not shown).

Immunogenic properties of rS protein

Sera from three rats immunized with Ac941S-infected cells by different routes were collected and examined for the presence of anti-TGEV neutralizing antibodies (Table 1). All animals elicited neutralizing antibodies, the highest titer (1:2500) being observed in rat 3 immunized by intraperitoneal injections.

DISCUSSION

In this paper we report the isolation and characterization of recombinant baculoviruses expressing either the complete coding sequence of the spike glycoprotein S of coronavirus TGEV or a truncated form without the transmembrane domain. The two recombinant proteins, called rS and rSt, respectively, were expressed by infected Sf9 cells with yields of approximately 1% of the total protein content. This yield is equivalent to that observed in TGEV-infected mammalian cells (data not shown). According to the available literature, membrane-anchored viral proteins expressed under the control of the polyhedrin promoter are produced at much lower levels than other proteins such as cytokines or nonglycosylated viral polypeptides (Luckow and Summers, 1988). In contrast, glycoprotein gIV of BHV1 (63K) was reported to be ex-

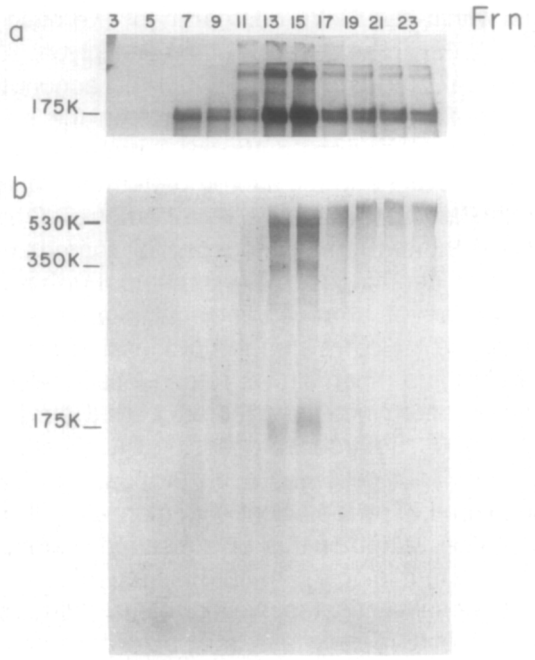

FIG. 4. Separation and identification of oligomeric forms of intracellular rS molecules. Cell extracts from Ac941S-infected Sf9 cells labeled with [^{35}S]methionine from 24 to 48 hr p.i. were fractionated by rate zonal centrifugation on a sucrose gradient. The gradient was harvested in 24 fractions (Frn) from the bottom (1) to the top (24). Numbered fractions were split into two parts, then either directly immunoprecipitated using mAb 3b5 (a) or crosslinked with DMS prior immunoprecipitation with the same mAb (b). S molecules were resolved by SDS–PAGE on an 8% (a) or 3.5% gel (b) and visualized following autoradiography.

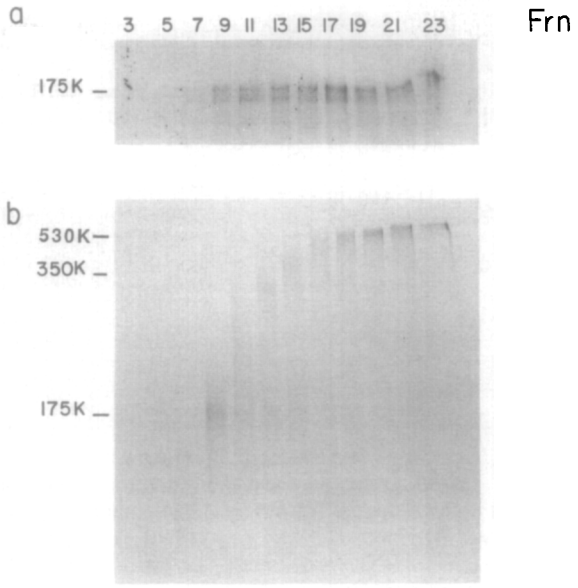

FIG. 5. Separation and identification of intracellular forms of anchor-free rSt molecules. Same legend as in Fig. 4.

pressed at a level close to that of the polyhedrin with the same pVL941-based vector as that used in our study (Van-Drunen Little-Van den Hurk *et al.*, 1991). The relatively low production of TGEV S we observed might thus reflect the inefficiency of lepidopteran insect cells to process proteins having such a great size (220K) and/or a high sugar content (28%). Consistent with this view is the finding that the transcription level of rS gene was only two- to fivefold lower compared to that of the polyhedrin gene (data not shown). Engineering the rS gene to produce an anchor-free molecule (rSt) with a reduced distance between the initiation ATG and the polyhedrin leader only slightly improved the level of expression.

A series of experiments was conducted to evaluate the extent of S protein maturation in insect cells. We first examined whether the process of oligomerization of the recombinant protein was similar to that of its authentic counterpart. We recently demonstrated that the spike is formed from a homotrimer of S molecules formed by oligomerization of the mannose-rich species 175K (called S′) in a *pre*-Golgi compartment; in addition, the oligomerization was shown to be a rate limiting step for S maturation (Delmas and Laude, 1990). Crosslinking experiments performed on the different rS forms separated by centrifugation established that the formation of highly stable S trimers actually occurred in insect cells. Using the same procedure, we failed to detect trimerized forms of the rSt species. However, intracellular rSt antigen was found to be recognized to some extent by mAbs directed against trimer-specific epitopes. From these observations we conclude that the absence of the membrane-anchoring region may affect the efficiency of trimerization perhaps due to the improper juxtaposition of rSt monomers except by ran-

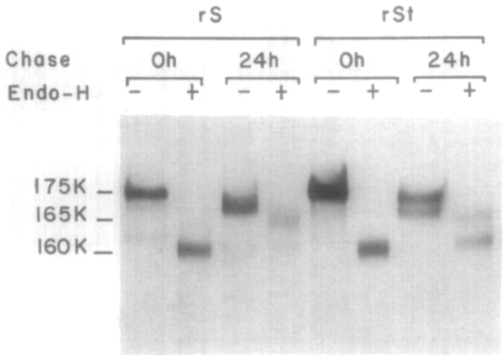

FIG. 6. Pulse-chase analysis and endo-H sensitivity of rS and rSt proteins synthesized in insect cells. Ac941S- or Ac941St-infected cells were pulse-labeled with [^{35}S]methionine for 1 hr at 24 hr p.i. and chased for the indicated period times. Polypeptides immunoprecipitated using mAb 3b5 were treated or mock-treated with endo-H, then resolved by SDS–PAGE on a 7.5% gel and visualized by fluorography.

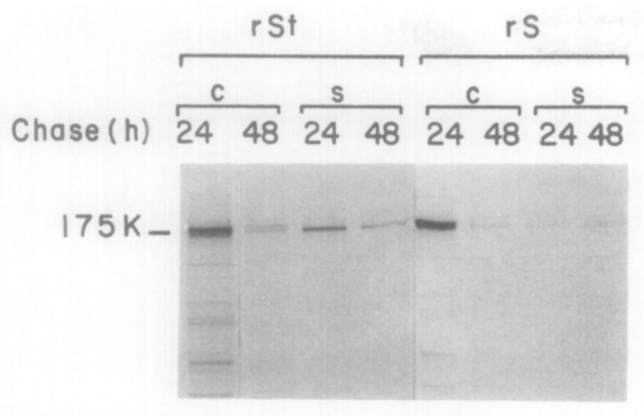

FIG. 7. Secretion of anchor-free rSt protein. Sf9 cells were infected with Ac941St or Ac941S and labeled at 24 hr p.i. with [^{35}S]methionine for 4 hr. Chase periods are indicated at the top of each lane. Polypeptides from cell lysates (c) and culture supernatants (s) were processed as in Fig. 6.

dom diffusion. Alternatively, the stability of rSt trimers may be suboptimal because like the HA protein trimer of influenza virus, an additional stability may be derived from the intramembranal segment (Doms and Helenius, 1986).

The rS protein appeared to undergo an incomplete carbohydrate processing in insect cells. Pulse-chase kinetics combined with endo-H glycosidase digestion allowed the characterization of two distinct intracellular species of rS: (i) a 175K species bearing oligosaccharide chains with endo-H sensitive linkages, and (ii) a "mature" species 170K exhibiting a partial endo-H resistance, assumed to result from a trimming of sugar chains within the Golgi compartment (Kornfeld and Kornfeld, 1985). The absence of terminal glycosylation in insect cells (Kuroda *et al.*, 1990) likely accounts for the marked discrepancy in Mr between the baculovirus-expressed and the authentic S proteins. Also of possible relevance to the altered pattern of glycosylation is the finding that a notable proportion of rS monomers and trimers formed nonspecific aggregates as revealed by centrifugation experiments. Furthermore, the presence of untrimed molecules after a 24-hr chase period indicates that the processing of rS protein occurs at a much slower rate in insect cells than previously determined in vertebrate cells (Delmas and Laude, 1990). Retarded processing of influenza HA protein in insect cells has been proposed to be the consequence of an inefficient oligomerization (Kuroda *et al.*, 1991). Whether this or subsequent steps are critical in determining the maturation rate of rS protein has not been investigated. Nevertheless, the retarded maturation of rS is likely to be the cause of its progressive appearance at the outer surface of the plasma membrane as evidenced by immunofluorescence studies.

The carbohydrate processing of anchor-free rSt molecules was found to be similar to that of rS with respect to the gradual conversion into an endo-H resistant, slightly faster migrating species. The process appeared to be somewhat delayed compared to rS, and this might be related to an impaired subunit assembly. In contrast to rS protein which remained cell-associated, rSt was secreted into the culture medium in a notable proportion. Among the two intracellular forms,

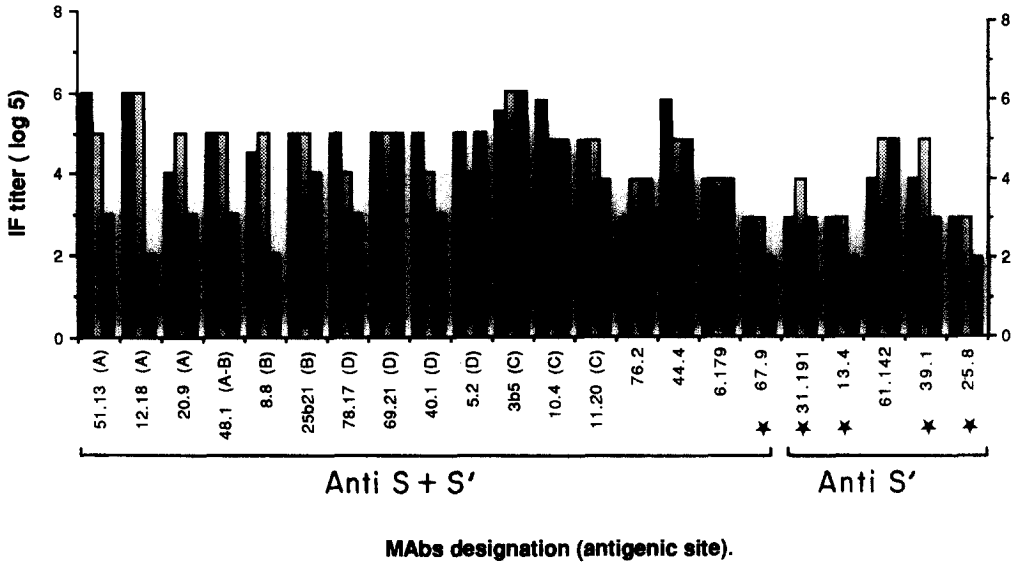

FIG. 8. Antigenic analysis of baculovirus-expressed S glycoproteins. TGEV-infected cells (■), Ac941S- (□) or Ac941St- (▨) infected Sf9 cells were fixed and subjected to an indirect IF assay using serial dilutions of the indicated mAbs. Antigenic sites are defined according to Delmas *et al.* (1986). S', see Fig. 2. *Trimer-specific mAbs.

TABLE 1

SEROLOGICAL RESPONSE OF RATS IMMUNIZED WITH AC941S RECOMBINANT BACULOVIRUS-INFECTED CELLS

Animal number[a]	Neutralization antibody titer at following day after primary immunization			
	0	40	80	116
1	<20[b]	—[c]	320	320
2	<20	640	—	640
3	<20	2500	—	2500

[a] Rats were immunized intradermally (1 and 2) or intraperitoneally (3) with 3×10^7 infected cells plus adjuvant and boosted two times by the same route. Determination of neutralizing antibodies titer was performed 12 days after boosting.

[b] Reciprocal of the highest dilution conferring resistance to viral cytopathic effect.

[c] (—) Not done.

only the low Mr species of rSt entered the exocytic pathway thus strengthening the view that it represented a Golgi-modified species.

The antigenic properties of rS protein were evaluated by analysis with a large panel of TGEV-specific mAbs. These mAbs have been shown to define four major antigenic sites clustered in the amino-half, presumably globular part of S molecule, as well as several unrelated epitopes including trimer-restricted epitopes (Delmas *et al.*, 1990; Delmas and Laude, 1990). The few differences detected between the baculovirus-expressed and the authentic proteins support our previous conclusion that terminal glycosylation is not required for acquisition of antigenicity by S protein (Delmas and Laude, 1991). The antigenicity of the cell-associated rS antigen was demonstrated by the anti-TGEV neutralizing antibodies found in immunized rats. The level of neutralizing antibodies observed in one rat immunized via the intraperitoneal route (Table 1, No. 3) was comparable to that found in pigs following recovery from natural infection with TGEV. In contrast to our results, a recent report examining the antigenicity of the baculovirus-expressed S protein of the murine coronavirus MHV-JHM failed to elicit neutralizing antibodies in immunized rats (Yoden *et al.*, 1989). There are no obvious explanations for the discrepancy in the two studies. The inefficient cleavage of MHV S protein in insect cells is unlikely of concern since the baculovirus-expressed uncleaved S species was shown to be recognized by neutralizing mAbs directed against the S1 (amino-half) subunit (Takase-Yoden *et al.*, 1990). Our own experiments led us to conclude that insect cells can provide an appropriate environment to yield a coronavirus spike with antigenic and immunogenic properties closely similar to those of its authentic homologue. An unexpected finding arose from the comparison of the antigenic reactivity of the complete and anchor-free recombinant S proteins. MAbs directed against the two sites A and B representing the major neutralization domain were shown to exhibit a significantly (25- to 100-fold) reduced reactivity towards rSt. This altered reactivity may result from the higher proportion of rSt in the form of aggregated monomers. Both A and B epitopes have been shown to be expressed prior to oligomerization of S protein (Delmas and Laude, 1990), but the reactivity of monomers and trimers has not been compared in quantitative terms. Alternatively, the cytoplasmic or more likely the transmembrane domain may control the "quality" of the contact between the spike subunits, so that their absence may result in subtle conformational changes. It would be of interest to determine whether an anchor-free TGEV S protein matured in mammalian cells would exhibit such an antigenic "restyling."

In conclusion, this study demonstrates that the baculovirus vector provides an appropriate system for expression of S protein in the absence of other TGEV components. Since the S protein is a likely candidate for inducing a protective immunity in infected swine such recombinant proteins may be useful tools for investigating aspects of the immune response to TGE.

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