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Conditional expression of the vesicular stomatitis virus glycoprotein gene in *Escherichia coli*

(lethal expression/signal sequence/expression plasmid/tryptophan operon/membrane protein)

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ABSTRACT **Bacterial plasmids that directed expression of the vesicular stomatitis virus glycoprotein (G-protein) gene under control of the tryptophan operon regulatory region were constructed. A plasmid directing the synthesis of a G-protein-like protein (containing the NH_2-terminal segment of seven amino acids encoded by the *trp*E gene fused to the complete G-protein sequence lacking only its NH_2-terminal methionine) could be transformed into *trp*R^+ (repressed) but not into *trp*R^- (derepressed) cells. This result suggested initially that derepressed synthesis of the G-protein-like protein encoded by this plasmid was lethal in *Escherichia coli*. Deletion of the sequence encoding the large hydrophobic segment near the COOH terminus of G-protein did not overcome this lethality. Lethality of derepressed synthesis was overcome by deletion of the G-protein gene region encoding 10 amino acids in the hydrophobic NH_2-terminal domain (signal peptide). Tryptic peptide mapping demonstrated that the G-protein-like protein and some truncated proteins encoded by the plasmid contained G-protein protein sequences. Antisera to vesicular stomatitis virus precipitated the G-protein-like protein, showing that it shares antigenic determinants with the authentic G-protein protein.**

Vesicular stomatitis virus (VSV), an enveloped animal virus, is the prototype of the rhabdovirus family (1). Although VSV causes a relatively mild disease of cattle, other members of the family such as rabies virus cause serious disease. Spikes on the surface of the VSV virion are composed of a single species of glycoprotein (G-protein). G-protein functions in the binding of virus to the host cell and therefore is essential for infectivity (2, 3). Antibodies directed against G-protein have been shown to neutralize virus infectivity (4). In the VSV virion, G-protein is anchored in the lipid envelope by a 20-amino acid hydrophobic transmembrane domain and a basic domain near the COOH terminus (5), leaving the NH_2-terminal 95% of G-protein exposed on the virion surface. Two complex oligosaccharides are apparently linked to asparagines at amino acid residues 178 and 335 (6–8).

As part of a determination of the nucleotide sequence of the VSV genome, we have isolated and determined the sequence of a cDNA clone containing the entire sequence coding for the VSV G-protein. This nucleotide sequence predicts a protein sequence of 511 amino acids, including a signal peptide of 16 amino acids that is not present on the mature protein (6).

Expression of eukaryotic viral glycoprotein sequences in bacteria may allow synthesis of large quantities of viral antigens that could be useful as vaccines. VSV should prove to be an especially useful model system for such expression studies because: (*i*) large amounts of viral glycoprotein can be prepared from virions, and its immunogenicity can be compared with that of the protein produced in bacteria; (*ii*) antibody neutralization of virus infectivity can be examined in a rapid plaque assay; and (*iii*) immunity to lethal VSV infection can be assayed readily in laboratory animals (9).

The preliminary expression studies reported here are a first step toward producing VSV glycoprotein antigens in bacteria. The results obtained show that it is necessary to modify the VSV gene G-protein so that its expression is not toxic to the cell. These results may be of general relevance to studies on expression of other viral glycoproteins.

MATERIALS AND METHODS

Materials. Restriction endonucleases and *Bal* 31 nuclease were purchased from Bethesda Research Laboratories. T4 polynucleotide kinase and bacterial alkaline phosphatase were from Boehringer Mannheim. T4 DNA ligase and DNA polymerase I (Klenow fragment) were from P-L Biochemicals [γ-^{32}P]ATP was from ICN.

Plasmids and Strains. The plasmid pAS621 is a pBR322 derivative carrying a 497-base pair *Hin*fI fragment containing the *trp* promoter, operator, leader, attenuator (poLa), and seven codons specifying the NH_2 terminus of *trp*E. The *Hin*fI-generated fragment was first converted to an *Eco*RI fragment by ligation to *Eco*RI linkers and then inserted into the *Eco*RI site of pBR322 to produce pAS620. One of the two *Eco*RI sites was eliminated as follows: pAS620 was partially digested with *Eco*RI, the linear pAS620 was isolated by agarose gel electrophoresis, and the *Eco*RI ends were filled in with the Klenow fragment of polymerase I. The blunt-ended linear pAS620 obtained was recircularized with T4 DNA ligase and used to transform *E. coli* strain C600. Two types of plasmids were recovered. One of these (pAS621) has a single *Eco*RI site downstream from the *trp* promoter and adjacent to the seventh codon in the *trp*E gene. Strain C600 YS1 is a minicell-producing strain (*thr, leu, ara, gal, lac*Y, *ton*A, *mal*A, *xyl, mtl, min*A, *min*B, *Endo*R^-). The *trp*R^- minicell-producing strain (AS10) is a *thr*$^+$ derivative of YS1 (unpublished data). The genotype of strain K802 is R_k^-, M_k^+, *gal*K, *su*II, *lac*Y, *met*.

Preparation of Minicells and Immunoprecipitation. Minicells were purified as described (10) through two cycles of sucrose gradient centrifugation and resuspended in 1 ml of minimal M9 salts supplemented with glucose, tryptophan (100 μg/ml), and the remaining amino acids except methionine (50 μM each). Cells were incubated at 37°C for 30 min (A_{540} = 1), labeled with 100 μCi (1 Ci = 3.7×10^{10} becquerels) of [^{35}S]methionine for 30 min at 37°C, and pelleted at 12,000 × *g* for 5 min. Samples of these cells to be analyzed directly were

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Abbreviations: VSV, vesicular stomatitis virus; G-protein, VSV glycoprotein.

boiled in sample buffer for 2 min prior to gel electrophoresis. Minicells used in immunoprecipitations were resuspended in 0.4 ml of 1% Nonidet P-40/1% sodium deoxycholate/0.1% $NaDadSO_4$/0.15 M NaCl, 0.01 M Tris·HCl, pH 7.2/1% Trasylol (FBA Pharmaceuticals) containing lysozyme at 1 mg/ml and incubated for 10 min at 4°C. The lysate was clarified by centrifugation for 10 min at 12,000 × *g*. Antibody (2 μl) was mixed with lysate and incubated at 4°C for 30 min. Subsequent antibody precipitation with fixed *Staphylococcus aureus* was as described (11), with *S. aureus* that had been preadsorbed with an *E. coli* lysate.

RESULTS AND DISCUSSION

A cDNA clone (pG 1) that contains a complete copy of the VSV G-protein mRNA coding sequences has been described; this clone was obtained by inserting a double-stranded DNA copy of the G-protein mRNA into the *Pst* I site of pBR322 by the homopolymer tailing procedure (6, 12). This cDNA insert had approximately 30 dG·dC residues adjacent to the *Pst* I sites at both ends. The dG·dC sequences at the 5′ end precede the natural initiation codon by 20 nucleotides. To prepare this insert DNA for expression experiments, we eliminated the dG·dC sequences by digestion of the insert DNA with *Bal* 31 nuclease, which digests both DNA strands at both ends of the insert DNA, leaving blunt ends. Molecules lacking approximately 50 nucleotides at each end were ligated to synthetic DNA fragments containing the *Eco*RI recognition site (*Eco*RI linkers) (Fig. 1). After digestion with *Eco*RI, these fragments were then inserted into the single *Eco*RI site of pBR325. Plasmids containing the *Eco*RI linkers preceding and following the initiator codon were isolated. The positions of the *Eco*RI linkers varied from 21 nucleotides before the initiator codon to 36 nucleotides after the initiator codon as determined by nucleotide sequence analysis. All plasmids retained the termination codon for the G-protein which was initially located 150 nucleotides from the 3′-terminal *Pst* I site.

Expression Strategy. To obtain expression of the VSV G-protein gene in *E. coli*, we used a plasmid (pAS621) containing the regulatory elements of the tryptophan (*trp*) operon and DNA encoding the first seven amino acids of anthranilate synthase, the product of *trp*E. This expression plasmid has a unique *Eco*RI site introduced just after this coding sequence (Fig. 1). Insertion of the VSV G-protein coding sequence at this site in the proper orientation and reading frame should result in synthesis of G-protein by read-through from the *trp*E fragment.

Initial experiments were performed with a G-protein insert that had an *Eco*RI linker positioned such that the G-protein sequence could be ligated in frame to the *trp*E segment in pAS621. This insert was ligated into the single *Eco*RI site of pAS621 and used to transform *E. coli*. A plasmid having the insert in the proper orientation for expression was identified by restriction endonuclease analysis. The structure of the NH_2-terminal coding portion from this plasmid (pGE1) is shown in Fig. 2. DNA of pGE1 was expected to encode a G-protein-like hybrid containing the first seven amino acids of anthranilate synthase, three amino acids encoded by the *Eco*RI linker, and the complete G-protein sequence except for the NH_2-terminal methionine.

Lethal Expression in *trp*R⁻ Cells. To examine the proteins produced by the pGE1 plasmid and their regulation by the *trp* repressor, we transformed *E. coli* minicell-producer strains carrying either the *trp*R⁺ (repressor positive) or *trp*R⁻ (repressor negative) alleles with pGE1 DNA and selected for ampicillin-resistant colonies. Transformation into the *trp*R⁺ strain gave the normal frequency of 5–10 × 10^5 colonies per μg of DNA,

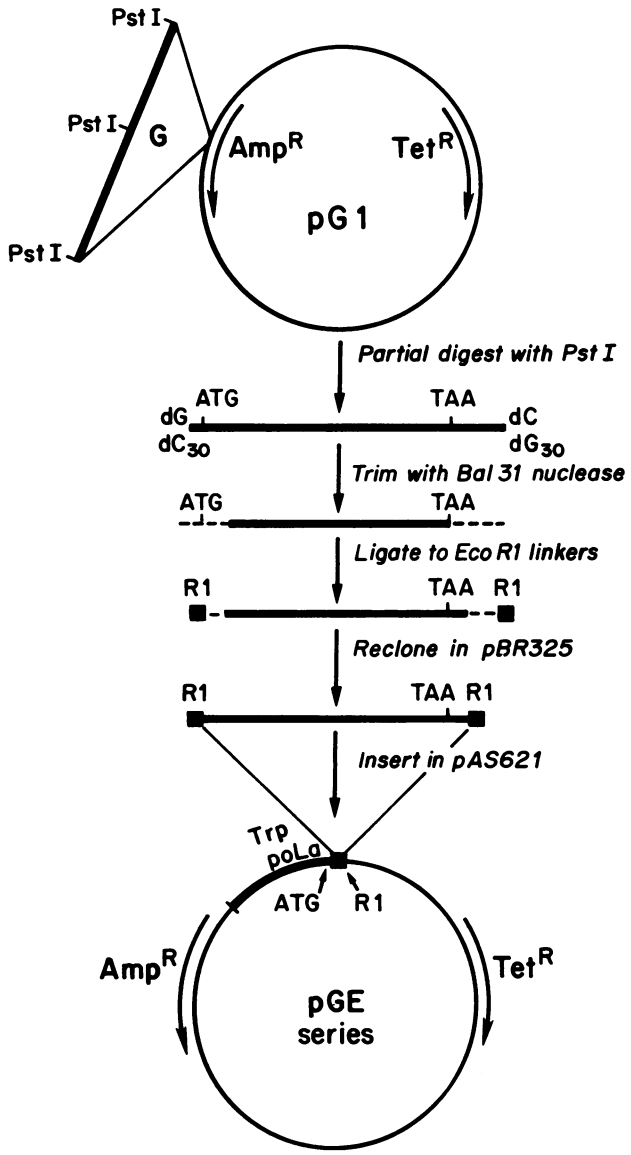

FIG. 1. Diagram of methods used to construct plasmids expressing VSV G-protein sequences. Partial digestion of pG1 DNA (100 μg) was for 15 min with 50 units of *Pst* I. The full-length insert (1 μg) was purified by electrophoresis on a 6% polyacrylamide gel followed by electroelution. *Bal* 31 digestion was at 20°C in 40 μl containing 20 mM Tris·HCl (pH 8), 12 mM $CaCl_2$, 12 mM $MgCl_2$, 0.3 M NaCl, 1 mM EDTA, 2 units of *Bal* 31, 1 μg of insert DNA, and 5 μg of pBR322 DNA fragments generated by *Hae* III cleavage. Samples (10 μl) were taken at 0, 2, 4, and 6 min and electrophoresed on a 6% acrylamide gel. The extent of digestion was monitored by the decrease in size of the *Hae* III fragments (*ca.* 15 nucleotides per min). The trimmed G-protein insert (6-min sample) was electroeluted from the gel and ligated to 50 pmol of *Eco*RI linkers. The reaction mixture was extracted with phenol and precipitated with ethanol; the precipitate was digested with 30 units of *Eco*RI at 37°C for 2 hr. After electrophoresis on a 6% polyacrylamide gel and electroelution, the G-protein insert containing *Eco*RI ends was ligated to 50 ng of pBR325 DNA (12) that had been digested with *Eco*RI and alkaline phosphatase. The DNA was then used to transform *E. coli* strain C600. Ampicillin-resistant chloramphenicol-sensitive colonies were screened for the presence of the insert DNA by digestion of small plasmid preparations (0.5 μg) with *Hae* III. Nucleotide sequences at the ends of 12 insert DNAs were determined by the chemical procedure (21). Appropriate inserts were then ligated to pAS621 DNA that had been linearized by *Eco*RI. These DNAs were used to transform *E. coli* strain K802. Ampicillin-resistant colonies were screened for the presence of the G-protein insert (21). The orientation of the G-protein insert relative to the *trp* control region was determined by digestion of plasmid DNA with *Hae* III.

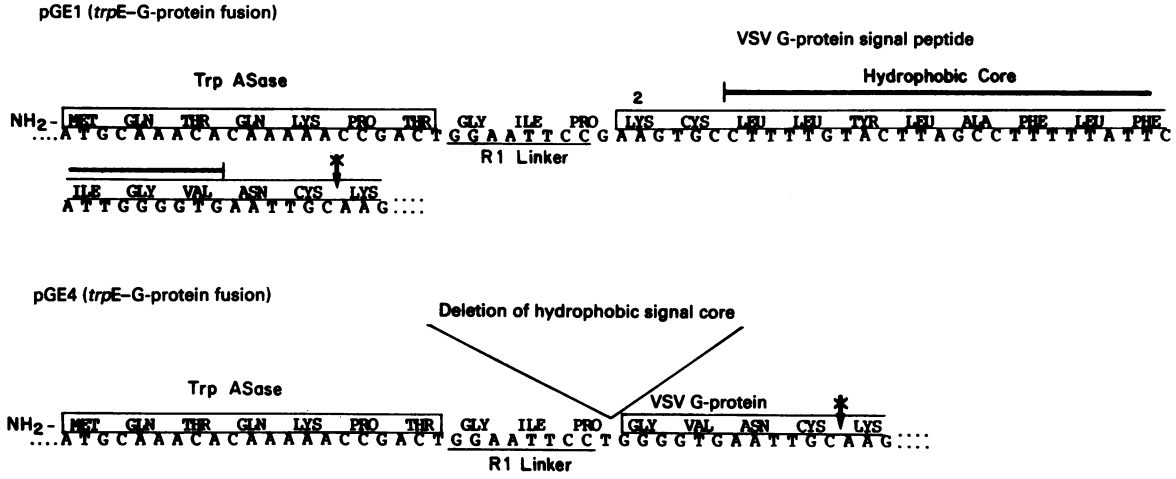

FIG. 2. Nucleotide and predicted protein sequences from plasmid regions encoding the NH_2-terminal regions of fusion proteins. The nucleotide sequences begin with residue 163 of the *trp* mRNA (23) encoding the NH_2 terminus of anthranilate synthase (ASase). VSV G-protein mRNA sequences begin following the *Eco*RI linkers. Arrow, point in the G-protein sequence where cleavage of the signal peptide occurs in animal cells.

whereas transformation into the *trp*R$^-$ strain yielded approximately 2 colonies per μg of DNA. Two plasmids recovered after transformation into the *trp*R$^-$ strain had deletions (>500 base pairs) which removed the *trp* control region and extended into the G-protein gene. This result suggested that synthesis of the G-protein-like sequence at a derepressed level was lethal. Consistent with this interpretation we found that a plasmid (pGE2) that had the same G-protein segment in the inverted orientation relative to pGE1 could be transformed into both *trp*R$^-$ and *trp*R$^+$ cells with normal frequency.

To determine if the *trp*R$^+$ strain containing pGE1 synthesized any G-protein-like protein, we used [^{35}S]methionine to label the proteins synthesized by minicells derived from this strain. Minicells synthesize only plasmid-encoded proteins (10, 13). Control cells carrying pAS621 synthesized two major bands of about 25,000 daltons which presumably were β-lactamase and its precursor (Fig. 3A). Cells containing pGE1 synthesize additional larger proteins including a major band of about 60,000 daltons migrating faster than the VSV G-protein marker. The G-protein-like fusion product would be expected to migrate faster than the glycosylated virion G-protein (14).

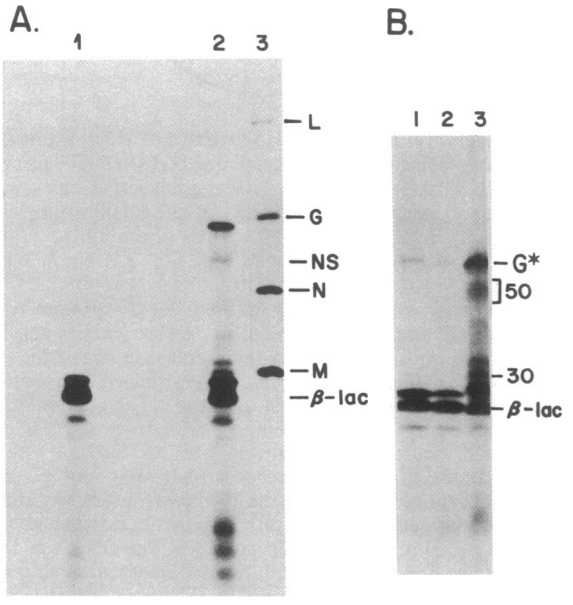

FIG. 3. Autoradiograms of proteins synthesized in *E. coli* minicells. (*A*) [^{35}S]Methionine-labeled proteins from *trp*R$^+$ minicells containing pAS621 (lane 1) or pGE1 (lane 2). Lane 3 shows ^{35}S-labeled marker proteins from VSV virions. (*B*) [^{35}S]Methionine-labeled proteins from *trp*R$^+$ minicells containing pGE1 (lane 1) or pGE4 (lane 2) or *trp*R$^-$ minicells containing pGE4 (lane 3). Electrophoresis was on 15% NaDodSO$_4$/polyacrylamide gels (24) in both *A* and *B*.

Basis of G-Protein Lethality. In addition to the hydrophobic signal sequence at the NH_2 terminus of the G-protein, there is a larger hydrophobic domain at the COOH terminus which spans the membrane in the VSV virion (5). We suspected initially that one or both of these domains might interfere with *E. coli* membrane function and could account for lethality of derepressed G-protein expression. To test this model, we first prepared a derivative of pGE1 in which the sequence encoding the COOH-terminal hydrophobic domain was deleted. DNA of pGE1 was digested with *Kpn* I which cuts uniquely in the G-protein gene (amino acid residue 336) and *Hin*dIII which cuts pBR322 DNA uniquely downstream from the G-protein gene (15). The deleted molecules were then treated with nuclease S1 to generate flush ends, recircularized with T4 DNA ligase, and used to transform *E. coli* strain K802. The plasmid obtained (pTG1) was expected to produce a truncated G-protein-like protein having the same NH_2 terminus as that produced by pGE1 but lacking the hydrophobic COOH terminus. This new protein was expected to terminate with three amino acids encoded by pBR322 sequences (15). Transformation of this DNA into *trp*R$^+$ and *trp*R$^-$ cells gave results identical to those obtained with pGE1, suggesting that the COOH-terminal domain was not responsible for the lethality.

We then examined the effect of deleting only the NH_2-terminal hydrophobic domain. A truncated G-protein gene that had been generated by *Bal* 31 exonuclease digestion and ligated to *Eco*RI linkers was inserted into the single *Eco*RI site of pAS621 and transformed into *E. coli* strain K802. The plasmid (pGE4), which had the proper reading frame (as determined by the nucleotide sequence) and orientation for expression, was obtained from this transformation. The NH_2-terminal structure of the G-protein-like protein which should be encoded by this plasmid is shown in Fig. 2. It is identical to the product specified by pGE1 except that 11 amino acids, including 10 from the hydrophobic core of the signal peptide, are deleted. In contrast to pGE1 and pTG1, transformation of pGE4 into both *trp*R$^+$ and *trp*R$^-$ cells occurred with high frequency (>5 $\times$ 10^5 col-

onies per μg of DNA). This result suggested that the lethal domain had been eliminated from the G-protein-like protein specified by pGE4.

To determine if a G-protein-like protein produced by pGE4 was in fact under *trp* regulation, we analyzed protein synthesis in *trp*R$^+$ and *trp*R$^-$ minicells carrying pGE4. The G-protein-like (G-protein*) proteins encoded by pGE1 and pGE4 in *trp*R$^+$ cells are shown in Fig. 3*B* (lanes 1 and 2). Expression of the pGE4-encoded protein was increased 15.2-fold relative to β-lactamase in the *trp*R$^-$ cells (lane 3). Also, other bands between G-protein* and β-lactamase were increased similarly, indicating that their synthesis was under *trp* regulation. For reasons that are not clear, the extent of derepression in minicells (*trp*R$^+$ vs. *trp*R$^-$) always was less than the 80-fold derepression observed in whole *E. coli* cells (refs. 16 and 17; unpublished data).

We observed consistently that the G-protein-like protein specified by pGE1 migrated marginally slower that that specified by pGE4. This difference is consistent with the expected deletion of 11 amino acids (Fig. 3*B*). This result also suggests that cleavage of the hydrophobic G-protein signal sequence is not occurring in the G-protein-like protein produced by pGE1. If cleavage had occurred at the normal site, then the product specified by pGE4 should be larger than that specified by pGE1. There is clear evidence that one eukaryotic signal sequence is cleaved properly in *E. coli* (18). The apparent failure to cleave the G-protein signal might result from the NH_2-terminal extension of 10 residues on the G-protein sequence. Failure to cleave this "hybrid" signal sequence could be responsible for the lethality of pGE1 in *trp*R$^-$ strains. The uncleaved NH_2 terminus might become permanently embedded in the *E. coli* membrane and interfere with membrane functions. It should be noted that even in *trp*R$^+$ cells the presence of pGE1 results in a growth rate only half as fast. Apparently even the low (repressed) level of synthesis of the G-protein-like protein is somewhat toxic. To examine cleavage (and possible toxicity) of the normal G-protein NH_2 terminus, it will be necessary to create a prokaryotic ribosome binding site adjacent to the G-protein initiator codon.

Peptide Mapping. To test the authenticity of the G-protein-like protein produced in minicells carrying pGE4 and to examine the nature of the smaller proteins made under *trp* control, we generated two-dimensional tryptic peptide maps of the full-size band (G-protein*) and the smaller proteins (designated 50 and 30 in Fig. 3*B*). The autoradiograms (Fig. 4) reveal nearly identical maps for [^{35}S]methionine-labeled G-protein* and authentic G-protein. The authentic G-protein from VSV virions is glycosylated and lacks the NH_2-terminal signal peptide. Because the glycosylated tryptic peptides in G-protein do not contain methionine (6), the G-protein peptides should be identical to those of G-protein* except for an additional NH_2-terminal peptide, if the NH_2 terminus of G-protein* is not cleaved. There are two peptides (arrows, Fig. 4) present in G-protein* but not in G-protein. These have mobilities consistent with their being the formylated and nonformylated NH_2-terminal fusion peptides from G-protein*. Tryptic maps of the smaller products show that these proteins contain subsets of the G-protein peptides including the presumed NH_2 terminus.

Level of G-Protein Expression. In minicells under dere-

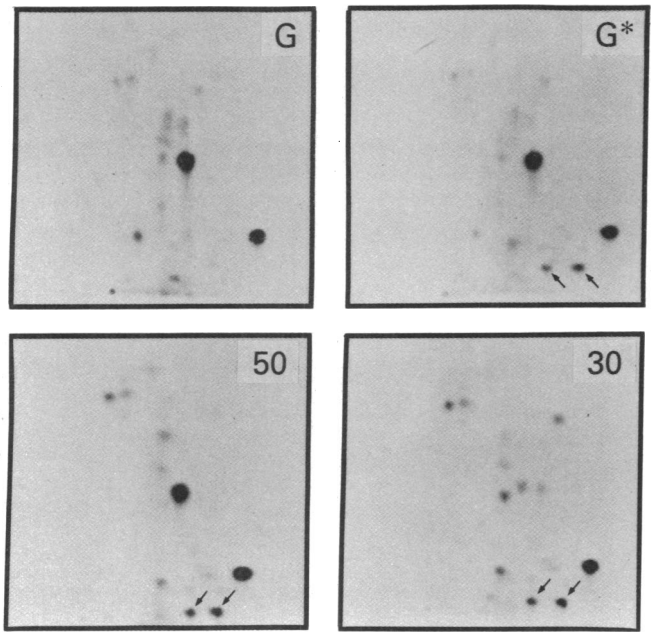

FIG. 4. Methionine-containing tryptic peptides of authentic G-protein and G-protein-related proteins synthesized in *E. coli* minicells. Electroelution of proteins from gels (25), digestion with trypsin, and two-dimensional separation were as described (11). The first dimension was electrophoresis in *n*-butanol/pyridine/acetic acid/H_2O, 2:1:1:36 (vol/vol), at pH 4.7; the second was chromatography in *n*-butanol/pyridine/acetic acid/H_2O, 97:75:15:60 (vol/vol), at pH 5.3.

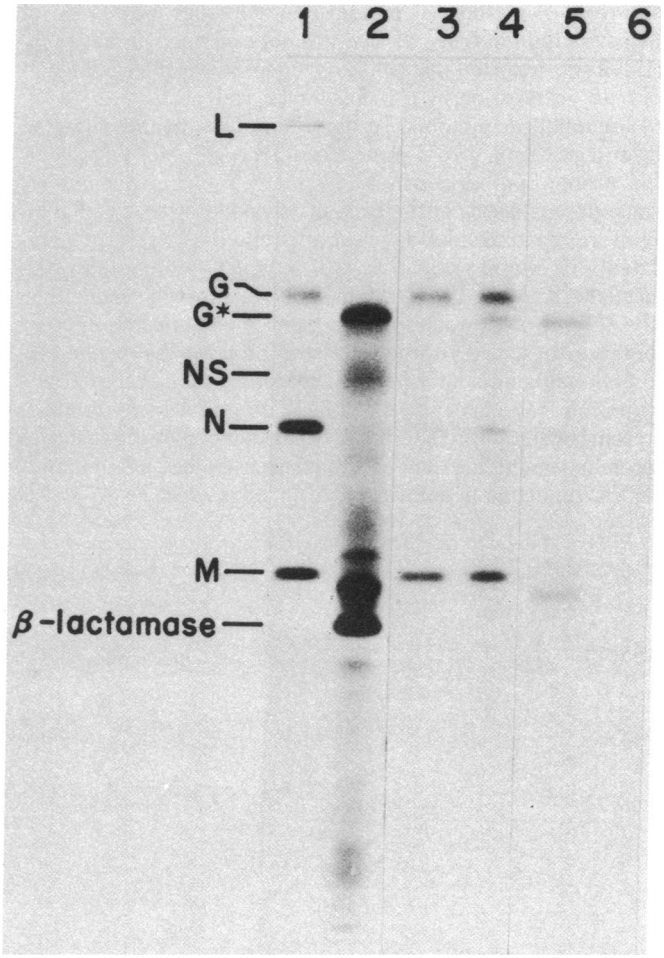

FIG. 5. Gel autoradiogram showing immunoprecipitates of ^{35}S-labeled proteins synthesized in *E. coli* minicells. Lanes: 1, total VSV virion protein markers; 2, total proteins synthesized in *E. coli* minicells containing pGE4; 3, immunoprecipitate of VSV virion proteins; 4, immunoprecipitate of VSV virion proteins combined with proteins from minicells containing pGE4; 5, immunoprecipitate of proteins synthesized in minicells containing pGE4; 6, same as lane 5 but with nonimmune serum.

pressed conditions (trpR$^-$ strain), the amount of labeled G-protein-like protein that accumulated during a 30-min labeling period was about half of the β-lactamase encoded by the same plasmid (pGE4). However, we have not been able to determine the amount of the G-protein-like protein that accumulated in whole *E. coli* cells. It was not detectable as a stained or labeled band over the background of cell protein (data not shown). From this result, we have concluded that the G-protein-like protein encoded by pGE4 constitutes less than 1% of the total cell protein. The G-protein-like proteins produced in minicells by pGE1 and pGE4 appear to be stable (data not shown); therefore, the low level of accumulation is probably not due to protein degradation. The G-protein fragments synthesized in minicells also appear to be stable and probably contain the NH_2 terminus of the fusion protein. They do not accumulate during a chase with unlabeled methionine, suggesting that they may result from premature termination during protein synthesis. We have not determined if such termination also occurs in whole *E. coli*.

The hemagglutinin of fowl plague virus has been expressed in *E. coli* from a plasmid containing the *trp* regulatory elements (19). This plasmid is similar to the one we have constructed, and it expressed this eukaryotic viral glycoprotein as 0.2%–0.7% of the total *E. coli* protein synthesized after induction. The *trp*E gene product expressed from a similar plasmid, however, constitutes 30% of the cell protein (17). Although expression of hemagglutinin in *trp*R$^-$ *E. coli* was not examined in that study (19), it was reported that cells grew more slowly after induction of *trp*R$^+$ expression by β-indoleacrylic acid.

Immunoprecipitation. To determine if antibodies directed against authentic VSV G-protein could recognize the G-protein-like protein synthesized in *E. coli* minicells, we carried out immunoprecipitations of the proteins encoded by pGE4. Rabbit antisera made against detergent-disrupted VSV (20) precipitated VSV proteins (Fig. 5, lane 3) and the G-protein-like product (lanes 4 and 5). Precipitation of several proteins smaller than the VSV G-protein-like protein (lane 5) presumably occurred because these are G-protein-related proteins that contain G-protein antigenic determinants. Demonstration that viral glycoprotein sequences produced in *E. coli* are actually useful as vaccines will require additional experiments showing that such proteins can induce virus neutralizing antibodies and immunity to VSV infection in animals.

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1. Wagner, R. R. (1975) in *Comprehensive Virology*, eds. Fraenkel-Conrat, H. & Wagner, R. R. (Plenum, New York), pp. 1–93.
2. Cartwright, B., Smale, C. J. & Brown, F. (1969) *J. Gen. Virol.* **5**, 1–10.
3. Bishop, D. H. L., Repik, P., Obijeski, J. F., Moore, N. F. & Wagner, R. R. (1975) *J. Virol.* **16**, 75–84.
4. Kelley, J. M., Emerson, S. U. & Wagner, R. R. (1972) *J. Virol.* **10**, 1231–1235.
5. Rose, J. K., Welch, W. J., Sefton, B. M., Esch, F. S. & Ling, N. C. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 3884–3888.
6. Rose, J. K. & Gallione, C. (1981) *J. Virol.* **39**, 519–528.
7. Etchison, J. R., Robertson, J. S. & Summers, D. F. (1977) *Virology* **78**, 375–392.
8. Reading, C. L., Penhoet, E. E. & Ballou, C. E. (1978) *J. Biol. Chem.* **253**, 5600–5612.
9. Jones, C. L. & Holland, J. J. (1980) *J. Gen. Virol.* **49**, 215–220.
10. Roozen, K. J., Fenwick, R. G. & Curtiss, R. (1971) *J. Bacteriol.* **107**, 21–23.
11. Sefton, B. M., Beemon, K. & Hunter, T. (1978) *J. Virol.* **28**, 957–971.
12. Bolivar, F., Rodriguez, R., Betlach, M. C. & Boyer, H. W. (1977) *Gene* **2**, 95–112.
13. Adler, H.I., Fisher, W. D., Cohen, A. & Hardigree, A. A. (1966) *Proc. Natl. Acad. Sci. USA* **57**, 321–326.
14. Knipe, D., Rose, J. K. & Lodish, H. F. (1975) *J. Virol.* **15**, 1004–1011.
15. Sutcliffe, J. G. (1979) *Cold Spring Harbor Symp. Quant. Biol.* **18**, 77–90.
16. Jackson, E. & Yanofsky, C. (1972) *J. Mol. Biol.* **69**, 307–313.
17. Hallewell, R. A. & Emtage, S. (1980) *Gene* **9**, 27–47.
18. Talmadge, K., Kaufman, J. & Gilbert, W. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 3988–3992.
19. Emtage, J. S., Tacon, W. C. A., Catlin, G. H., Jenkins, B., Porter, A. G. & Carey, N. H. (1980) *Nature (London)* **283**, 171–175.
20. Fan, D. P. & Sefton, B. M. (1978) *Cell* **15**, 985–992.
21. Maxam, A. M. & Gilbert, W. (1980) *Methods Enzymol.* **65**, 501–561.
22. Grunstein, M. & Hogness, D. S. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 3961–3965.
23. Lee, F., Bertrand, K., Bennett, G-protein. & Yanofsky, C. (1978) *J. Mol. Biol.* **121**, 193–217.
24. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–684.
25. Welch, W., Sefton, B. M. & Esch, F. (1981) *J. Virol.* **38**, 968–972.