JOURNAL OF VIROLOGY, July 1990, p. 3486–3491
0022-538X/90/073486-06$02.00/0

Vol. 64, No. 7

Localization of the Membrane-Associated Region of Vesicular Stomatitis Virus M Protein at the N Terminus, Using the Hydrophobic, Photoreactive Probe ^{125}I-TID

JOHN LENARD* AND ROGER VANDEROEF

Department of Physiology and Biophysics, Robert Wood Johnson Medical School (at Rutgers), University of Medicine and Dentistry of New Jersey, 675 Hoes Lane, Piscataway, New Jersey 08854-5635

Received 6 February 1990/Accepted 16 April 1990

The membrane-reactive, photoactivatable probe ^{125}I-TID {3-(trifluoromethyl)-3-(*m*-[^{125}I]iodophenyl)-3H-diazirine} was found to label the M protein of vesicular stomatitis virus about 40% as much as G protein in intact virions, in agreement with labeling studies with other probes. By analyzing limited tryptic digestion and specific chemical cleavage products, the label was essentially entirely localized within the first 19, and probably within the first 5 to 10, amino acid residues at the N terminus, identifying this short amphipathic segment as the likely site of interaction of M protein with the viral bilayer.

Vesicular stomatitis virus (VSV) is an enveloped virus consisting of an RNA genome of negative sense, five viral proteins, and membrane lipids derived from the host cell. Electron micrographs show that the virus possesses a regular, bulletlike shape, with dimensions of ca. 80 by 170 nm. Three of the five viral proteins, G, M and N, are regarded as structural and together account for >90% of total virion protein. Of these three, the dispositions of two, G and N, are well established: G is a glycoprotein which spans the bilayer membrane once and has most of its mass oriented externally; N is the ribonucleoprotein, which interacts with the viral genome RNA to form an ordered, RNase-resistant structure that comprises the only template recognized by the viral RNA-dependent RNA polymerase (7).

In contrast, much less is known about the disposition of the M protein. M is required for viral assembly and budding, since viral strains carrying temperature-sensitive lesions in M protein do not form mature viral particles at the restrictive temperature, even though normal (or excessive) amounts of viral genome RNA and proteins are present in the infected cell (6, 20, 23). M protein is thought to promote assembly by interacting with an assembled N protein-genomic RNA complex on the one hand and a portion of the host cell plasma membrane containing G protein on the other (12, 24, 32). Of these, the interaction between M and N proteins appears more specific than that between M and G proteins: infectious virions produced under a variety of conditions contain invariant M/N ratios while differing more than six-fold in their M/G ratios (11).

Several lines of evidence suggest that M protein associates with the viral bilayer at or near its surface but does not penetrate deeply: (i) the reaction of intact virions with two different hydrophobic, photoactivatable probes (iodonaphthylazide or pyrenesulfonylazide) resulted in substantial labeling of M protein, in addition to the expected labeling of lipids and G protein (12, 34); (ii) M protein was cross-linked to phospholipid acyl chains by water-soluble cross-linkers but not by hydrophobic cross-linkers that partition into the membrane interior (22, 34); (iii) a photoactivatable group located on the acyl chain of a phospholipid that was metabolically incorporated into VSV virions could label G but not M protein (29). This disposition of M protein near the bilayer surface is consistent with its deduced amino acid sequence, which reveals no long hydrophobic stretches suggestive of transmembrane regions (15, 25).

The photoactivatable compound 3-(trifluoromethyl)-3-(*m*-[^{125}I]iodophenyl)-3H-diazirine (^{125}I-TID) has been reported to possess several general advantages over previously used membrane probes, such as those referred to above. Advantages include stability and activation by longer-wavelength (lower-energy) light. Most important, however, is the lack of selectivity of ^{125}I-TID in forming covalent bonds upon photoactivation. This has permitted the identification in certain proteins of individual amino acid residues that penetrate the bilayer, a degree of resolution that cannot be obtained with probes that retain some preference for reaction with specific functional groups (3). Thus, reaction of two different proteins (bacteriorhodopsin and influenza hemagglutinin) with ^{125}I-TID resulted in the preferential labeling of only those hydrophobic amino acids that were thought to constitute the membrane-penetrating face of an amphipathic helix (4, 8).

In this report, we have studied the reaction of ^{125}I-TID with VSV virions. As expected from the above considerations, the reaction showed less preference for protein functional groups than did previously used probes; nonetheless, M protein was substantially labeled. Analysis of specific cleavage products of M protein localized the ^{125}I-TID-reactive region to the first few amino acids at the N terminus.

MATERIALS AND METHODS

Cells and viruses. The Orsay wild type and *ts*O23(M) mutant of VSV Indiana were originally obtained from A. Huang. They were grown in BHK-21 cells and purified as described previously (14). The wild type was grown at 37°C, and *ts*O23 was grown at 31°C.

Reagents. ^{125}I-TID was purchased from Amersham Corp., Arlington Heights, Ill. All other reagents were of the best available grade, generally purchased from Sigma Chemical Corp., St. Louis, Mo.

^{125}I-TID labeling. ^{125}I-TID (100 to 250 μCi) was added to purified VSV (15 to 30 mg of viral protein) in 1 to 2 ml of phosphate-buffered saline. For illumination, the mixture was

* Corresponding author.

placed in a Pyrex test tube (Corning Glass Works, Corning, N.Y.) mounted in a Pyrex beaker containing water at room temperature, thus providing both temperature regulation and a low-wavelength (<290 nm) filter. Illumination was carried out for 3 to 5 min with stirring, with a focused beam from a high-pressure mercury arc lamp (UVP Inc., San Gabriel, Calif.; kindly loaned by James E. Rothman, Princeton University, Princeton, N.J.). The labeled virus was washed to remove excess label by pelleting twice from phosphate-buffered saline.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). SDS-PAGE was done by the method of Laemmli (10), with acrylamide concentrations of either 10 or 18%, as indicated below. ^{125}I-TID labeling was determined by gamma counting of gel slices. Protein concentrations were estimated from slices of Coomassie blue-stained gels by extraction of the Coomassie blue with aqueous isopropanol-SDS and determination of the optical density at 595 nm (1).

Virus fractionation. ^{125}I-TID-labeled virus was disrupted in 0.5% Triton X-100 in low-salt buffer (10 mM Tris, 10 mM NaCl, pH 8.1) and centrifuged on a discontinuous 35 to 65% sucrose gradient (2 h, 280,000 × *g*). Under these conditions, most of the viral G protein and lipid (containing most of the radioactive label) remained at the top of gradient, while the rest of the viral components (the M-N-RNA-enriched fraction) sedimented at the 35 to 65% sucrose interface or at the bottom of the tube.

Trypsin cleavage. M protein present in the M-N-RNA-enriched fraction prepared from labeled virions was cleaved by acetylated trypsin (20 μg/ml) in 550 mM NaCl as previously described (21).

M protein. The M-N-RNA-enriched fraction was subjected to preparative-scale SDS-PAGE. The M protein band was located after staining with Coomassie blue, sliced out of the gel, and stored frozen until needed.

Formic acid cleavage. Slices (1 to 2 cm) of the gel strip containing M protein were subjected to selective hydrolysis by formic acid at the Asp-Pro linkage of the protein and reelectrophoresed by 18% SDS-PAGE as described elsewhere (28).

RESULTS

^{125}I-TID labeling of VSV. Purified VSV was labeled with ^{125}I-TID as described above and washed, and the components were separated by 10% SDS-PAGE. The results are shown in Fig. 1. Most of the counts associated with the virus migrated with lipids at the dye front (beyond fraction 40), while viral G and M proteins were also significantly labeled. N protein was only very slightly labeled, as expected for a protein that does not penetrate membranes. Table 1 shows the distribution of ^{125}I-TID label in the different viral components, as compared with previously published values for labeling with iodonaphthylazide (12). It can be seen that relative to labeling of the transmembrane G protein, lipids are much more and N proteins much less labeled by ^{125}I-TID than by iodonaphthylazide. This further supports the idea (indicated also by comparative studies on erythrocyte membranes; 3) that ^{125}I-TID is a less-specific and therefore more-useful membrane probe than iodonaphthylazide, i.e., it has less selectivity for reaction with particular functional groups in proteins.

In previous iodonaphthylazide-labeling experiments, it was observed that temperature-sensitive M proteins in VSV virions were labeled two to three times as much as M protein in wild-type virions (Table 1) (12). Accordingly, it was of interest to compare wild-type and *ts*O23 virions with ^{125}I-TID labeling. As shown in Table 1, however, the distribution of ^{125}I-TID between the components of wild-type and *ts*O23 virions was essentially identical.

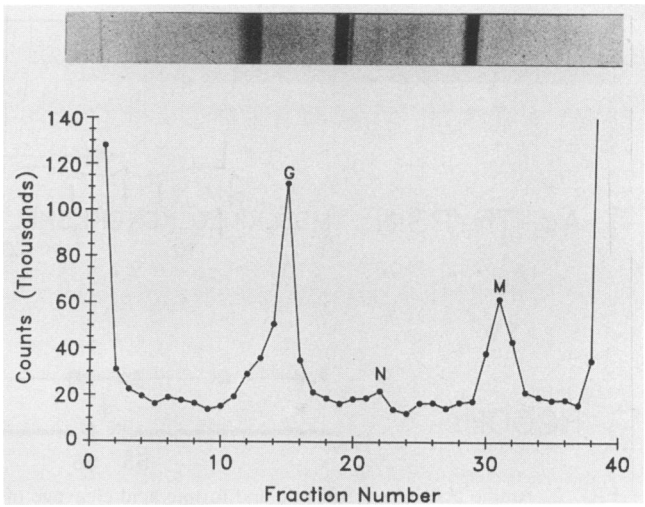

FIG. 1. SDS-gel electrophoresis of ^{125}I-TID-labeled VSV. The Coomassie blue-stained gel is shown at the top, and the distribution of counts is shown below.

Localization of ^{125}I-TID on M protein. M protein was cleaved in two different ways to determine the location of ^{125}I-TID label on the polypeptide chain (Fig. 2). Specific, stepwise removal of the N-terminal 44 residues by acetyl trypsin occurs from undenatured protein (16, 19); after SDS treatment, acetyl trypsin cleaved M into numerous small fragments (data not shown). Cleavage with formic acid, on the other hand, could be successfully carried out on SDS-denatured M protein contained in gel slices.

Results of limited proteolysis of the M-N-RNA-enriched fraction are shown in Fig. 3 and 4. After 7 min of treatment with acetyltrypsin, two new bands appeared, both of which lacked appreciable amounts of label. One band migrated almost identically with M protein, and the other migrated at about 24 kilodaltons (kDa). Both may be assigned as arising from M protein because (i) neither N nor G protein bands were appreciably diminished in amount, even after 60 min of proteolysis and (ii) a 24-kDa fragment of M was expected after brief trypsinization, since this has been shown previously to be an intermediate in the cleavage of M protein by acetyltrypsin and to arise from cleavage after residues 18 or 19 (19, 21). The slower band has not previously been observed, but examination of the sequence (Fig. 2) makes it

TABLE 1. Distribution of ^{125}I-TID and iodonaphthylazide after reaction with wild-type and *ts*O23 VSV

Viral component	Relative distribution[a] of:			
	^{125}I-TID in:		Iodonaphthylazide[b] in:	
	Wild type	*ts*O23	Wild type	*ts*O23
G protein	1	1	1	1
M protein	0.44	0.40	0.40	1.19
N protein	0.05	0.06	0.35	0.37
Lipid	15.6	15.6	4.8	ND

[a] Relative to G protein.
[b] See reference 12. ND, Not determined.

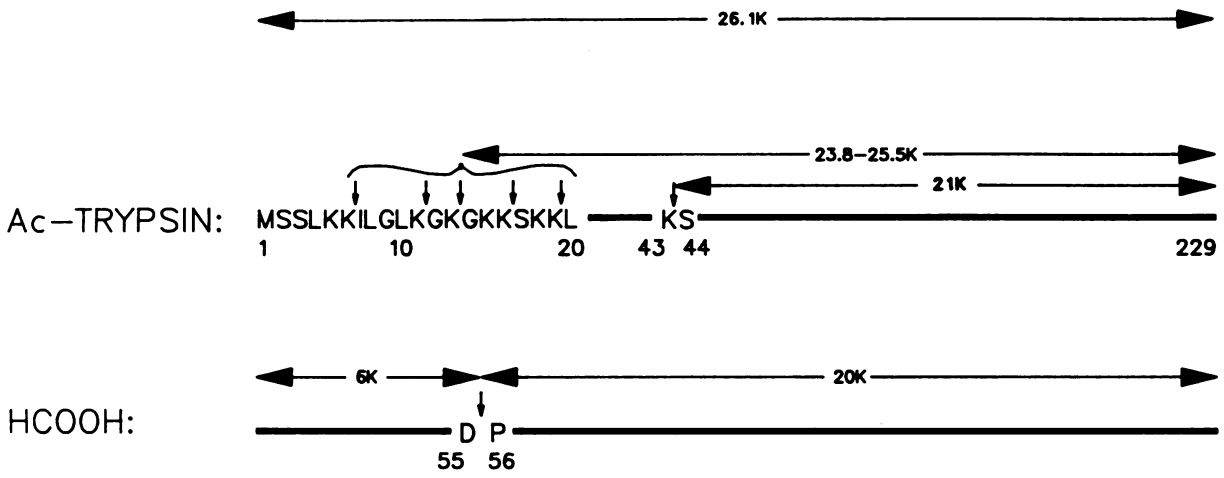

FIG. 2. Amino acid sites of tryptic and formic acid cleavage of M protein. Molecular weights (in thousands [K]) are given for indicated fragments.

reasonable to suggest that it arises from cleavage after residues 5 or 6 (Fig. 2). It is quite striking that even this largest M-protein fragment was essentially devoid of label, thus localizing most of the label to the extreme N terminus of the protein. After 60 min of incubation with acetyltrypsin, both of these bands have disappeared and been replaced by a band of 21 kDa, which corresponds to the well-characterized trypsin-resistant core of M protein (16, 19, 21). The progressive disappearance of the two bands seen after 7 min and the increase in the appearance of the 21-kDa band at 60 min are diagnostic of a precursor-product relationship, confirming the identity of the bands. It is noteworthy also that after 7 and 60 min of digestion, a progressive increase was seen in a lightly Coomassie blue-staining band containing substantial label, which moved slightly more slowly than did residual unextracted lipid.

This localization was confirmed by a further experiment using M protein that had been gel purified from the M-N-RNA-enriched fraction. If the M protein band was cut out of a gel such as that shown in Fig. 3 (left lane) and reelectrophoresed on an 18% gel, a single radiochemically pure band containing no residual unextracted lipid was seen (data not shown). Cleavage by formic acid occurred preferentially at Asp-Pro linkages, which occur only once in M protein, at residues 55 and 56 (Fig. 2). Formic acid cleavage in a gel slice followed by SDS-PAGE in an 18% gel yielded two fragments of the expected sizes, of which the smaller fragment contained >90% of the label (Fig. 5).

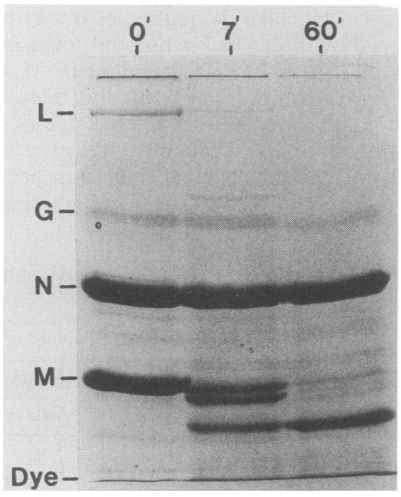

FIG. 3. Coomassie blue-stained gels of M-N-RNA-enriched fraction extracted from ^{125}I-TID-labeled VSV after treatment with acetylated trypsin (as described in Materials and Methods) for the indicated times in minutes.

Mass spectrometry of the amino-terminal peptide. Since the above results localized the ^{125}I-TID-reactive region of M protein to the first few residues at the amino terminus, we attempted to characterize this region further using mass spectrometry. Previous attempts to sequence this protein had led to the conclusion that the terminus was blocked (D. D. Sabatini, personal communication). It was therefore impossible to identify the individual ^{125}I-TID-labeled amino acids by Edman degradation on conventional sequenators, as had been successfully done for other ^{125}I-TID-labeled proteins (4, 8).

Mass spectrometry has been successfully used to elucidate the structure of blocked N-terminal peptides. Of especial interest, three cytoplasmically synthesized, enveloped virus proteins (the N protein of VSV and the M and NP proteins of Sendai virus, a paramyxovirus) were all found to have lost their initiator methionine residues and to have their N termini blocked by acetyl groups (2, 26).

In the present case, mass spectrometry was not expected to be able to identify the ^{125}I-TID-labeled residues, since labeling is always sub-stoichiometric and since it may be assumed from the lack of selectivity in labeling that several different adducts are present at each residue. The structure of the unlabeled N terminus was of interest, however, in light of the labeling pattern. Accordingly, supernatant obtained after trypsin treatment of unlabeled M-N-RNA was analyzed by fast atom bombardment mass spectrometry. A major peak was found at an M + 1 of 476, which could be assigned to the structure Ac-Ser-Ser-Leu-Lys-COOH, where Ac is acetyl. This corresponds to the N-acetylated form of the first 4 residues after the initiator methionine, as deduced from the cDNA sequence of the M protein gene (14, 23). An additional major peak was found at an M + 1 of 543, which could not be assigned. Of particular interest for the

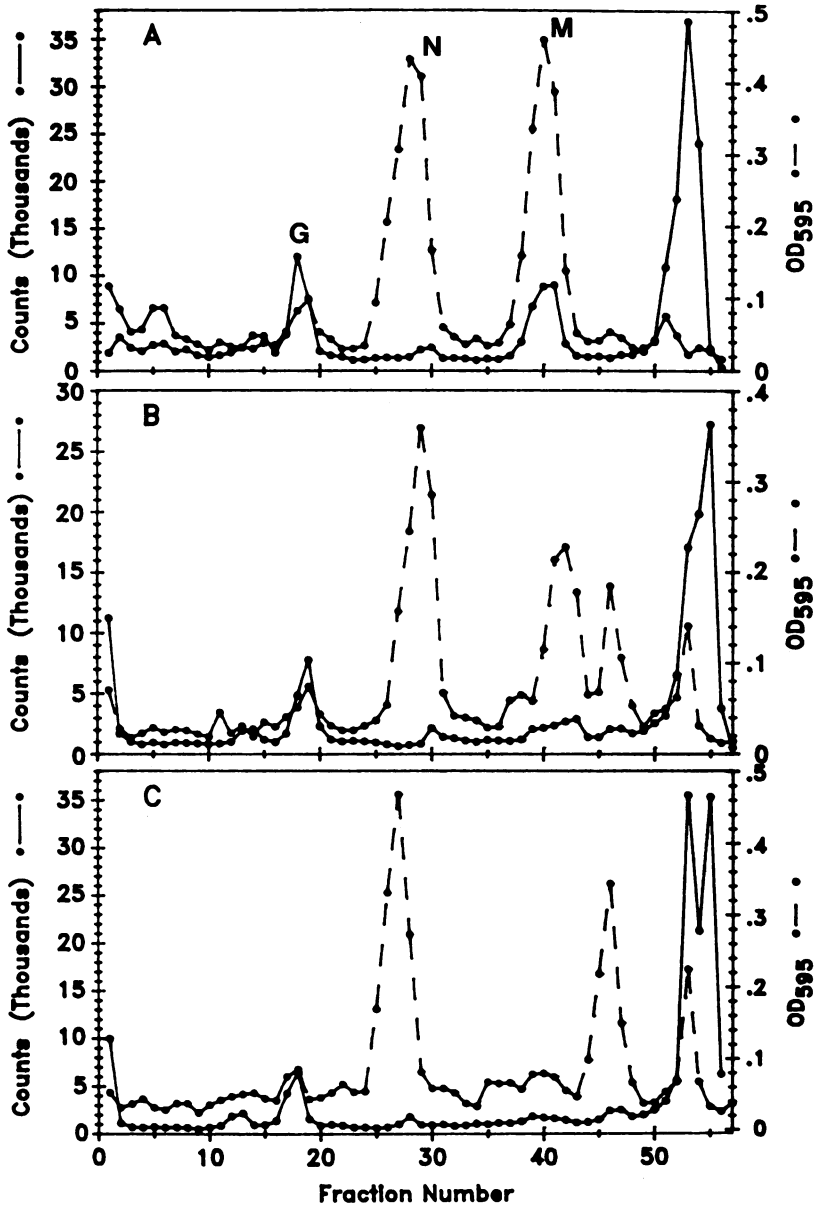

FIG. 4. Distribution of counts and Coomassie blue-staining material in the gels shown in Fig. 3. (A) 0 min; (B) 7 min; (C) 60 min. OD_{595}, Optical density at 595 nm.

present study, however, was that no evidence was found to indicate the presence of a covalently attached fatty acid in the N-terminal region.

DISCUSSION

With this report ^{125}I-TID becomes the third photoactivatable membrane probe to label M protein in intact VSV virions to a significant degree. All three probes introduced at least 40% as much label into M protein as into G protein, which is an integral protein known to cross the membrane just once. This concordance strengthens the suggestion that M protein does penetrate the viral membrane to some extent. The previous suggestion that mutant M proteins might interact more intimately than wild-type M proteins with viral membranes (12) could not be confirmed by using ^{125}I-TID (Table 1).

In contrast, the amount of label introduced into N protein by the three probes varied considerably: ^{125}I-TID and naphthalenesulfonylazide labeled N only slightly, while a significant amount of labeling occurred with iodonaphthylazide (Table 1; 12, 34). N protein is thought not to interact with the viral envelope directly (11, 12, 24, 32, 34), although a contrary view has also been suggested (18). The very much larger lipid/protein-labeling ratio obtained with ^{125}I-TID than with iodonaphthylazide (Table 1) supports a previous suggestion (3) that ^{125}I-TID is less specific in its reactivity and is hence more generally useful as a probe of membrane penetration than is iodonaphthylazide.

While ^{125}I-TID and other hydrophobic probes have an extensive and generally successful record of labeling membrane-penetrating proteins (3), they can label lipid-free hydrophobic pockets of proteins as well. For example, ^{125}I-

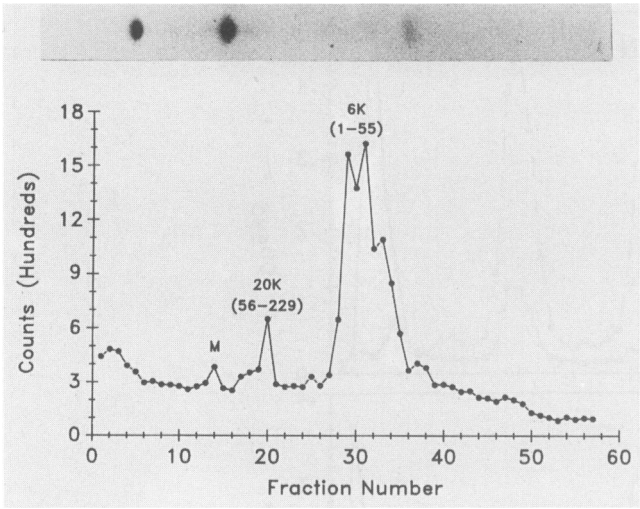

FIG. 5. SDS-gel electrophoresis of ^{125}I-TID-labeled M protein after cleavage with formic acid. The Coomassie blue-stained gel is shown at the top, and the distribution of counts is shown below. Numbers in parentheses refer to amino acid residues.

TID has been reported to label the phenothiazine binding site of calmodulin in a calcium-dependent manner (5, 9). The existence of such a hydrophobic pocket in M protein cannot be ruled out as an explanation of our results. However, several considerations suggest that this is unlikely. (i) There is no evidence that M protein has a binding site for hydrophobic substances. (ii) M protein must interact with the membranes of virions and infected cells in order to perform its assembly function. (iii) The region of M protein that is labeled is amphipathic rather than hydrophobic. The best interpretation of these results, therefore, is that ^{125}I-TID is labeling a membrane-penetrating segment of the M polypeptide chain.

The ^{125}I-TID in the M protein was localized with highest resolution after brief exposure (7 min) to acetyl trypsin (Fig. 3 and 4B). A band was formed that migrated almost identically with uncleaved M but which has lost essentially all of its label. If it is assumed that the faster-migrating band in the same lane corresponds to cleavage after position 18 or 19 (19; Fig. 2), then the slower band was probably formed by cleavage after positions 5 and 6; cleavage after position 10 (the next trypsin-sensitive site along the chain) would generate a species differing by over 1 kDa from uncleaved M, which should be better separated. The most likely location of the label, and therefore of the membrane-penetrating region of the protein, is thus within the N-terminal tetrapeptide, probably comprising Ac-Ser-Ser-Leu-Lys.

This is a surprising result, since it appears that the N terminus of M protein is not very hydrophobic; on the contrary, 8 of the 15 residues in positions 5 to 19 are lysines (Fig. 2). Furthermore, M protein, in contrast to G protein, contains no incorporated palmitic acid (27) or myristic acid (D. Lyles, personal communication), and no evidence of other hydrophobic modification has been reported. In fact, the mass spectrometry results reported here provide positive evidence that the first 4 residues are not modified, except for the introduction of an acetyl group at the N terminus.

Ogden et al. (19) found that purified M protein bound to acidic phospholipid vesicles equally well regardless of whether the 43 (or 44) residues of the N terminus had been removed by trypsin. However, M protein is a very basic protein, and polycations of all kinds bind strongly to anionic phospholipid vesicles; the specificity of the M protein-phospholipid interaction was not demonstrated by a functional assay. In light of the present findings, the relevance of this interaction (19, 30, 31, 33) to the actual viral budding process must be questioned.

One possible explanation of the observed results is suggested when the first 18 residues of M protein are placed on a helical wheel (data not shown). It was observed that the leucines and isoleucine at positions 4, 7, 8, and 10 (Fig. 2) were all on one side of the wheel and could thus compose the hydrophobic face of a short amphipathic helix; most of the lysine residues compose the other half of the wheel. Two early published observations are consistent with the idea that M protein interacts with the bilayer through a lysine-containing amphipathic structure of this sort. (i) In a careful comparison between the phospholipid contents of VS virions and host cell plasma membranes, McSharry and Wagner showed that the anionic lipids phosphatidylserine, phosphatidylinositol, and phosphatidic acid were enriched 1.4- to 3.3-fold in virions as compared with plasma membranes (13). (ii) Mudd observed that treatment of VS virions at pH 11 resulted in the clean dissociation of a sheet of membrane containing only G protein and lipids from the underlying viral structure (17).

Sequence analysis suggests some limitations to this hypothetical bilayer-associated amphipathic region. Analysis of temperature-stable revertants of the temperature-sensitive M protein mutant *ts*O89 showed that a Ser→Pro change at position 3 (Fig. 2; position 2 from the N terminus) was compatible with wild-type function (15); hence, the N-terminal Ac-Ser-Ser cannot be functionally required in such a hypothetical helix. On the other end, the very hydrophilic nature of residues 11 to 19 would seem unsuitable for an amphipathic structure. Nonetheless, our results permit the testable prediction that the N-terminal region of 5 to 10 amino acids is responsible for bilayer anchoring of the M protein and is therefore required for budding.

It should be noted that these experiments address only the question of how (and whether) M protein interacts with the lipid bilayer of the viral envelope. They provide no information regarding any interaction of M protein with the cytoplasmic portion of G protein. If such an interaction occurs, it could involve the sequence immediately adjacent to the bilayer-penetrating region, or the M protein might loop out and interact with G elsewhere on its sequence, yielding a two-point attachment to the membrane.

LITERATURE CITED

1. **Ball, E. H.** 1986. Quantitation of proteins by elution of Coomassie brilliant blue R from stained bands after sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Anal. Biochem. **155**:23–27.
2. **Blumberg, B. M., K. Rose, M. G. Simona, L. Roux, C. Giorgi, and D. Kolakofsky.** 1984. Analysis of the Sendai virus M gene and protein. J. Virol. **52**:656–663.
3. **Brunner, J.** 1989. Photochemical labeling of apolar phase of membranes. Methods Enzymol. **172**:628–687.
4. **Brunner, J., A. J. Franzusoff, B. Luscher, C. Zugliani, and G. Semenza.** 1985. Membrane protein topology: amino acid residues in a putative transmembrane a-helix of bacteriorhodopsin labeled with the hydrophobic carbene-generating reagent 3-(trifluoromethyl)-3-(m-[125I]iodophenyl)diazirine. Biochemistry **24**:5422–5430.
5. **Buerkler, J., and J. Krebs.** 1985. Structural studies of calmodulin and related calcium-binding proteins by hydrophobic labeling. FEBS Lett. **182**:167–170.
6. **Clinton, G. M., S. P. Little, F. S. Hagen, and A. S. Huang.** 1978.

The matrix (M) protein of vesicular stomatitis virus regulates transcription. Cell **15**:1455–1462.

7. **Emerson, S. U.** 1985. Rhabdoviruses, p. 1119–1132. *In* B. N. Fields (ed.), Virology. Raven Press, New York.
8. **Harter, C., P. James, T. Bachi, G. Semenza, and J. Brunner.** 1989. Hydrophobic binding of the ectodomain of influenza hemagglutinin to membranes occurs through the "fusion peptide." J. Biol. Chem. **264**:6459–6464.
9. **Krebs, J., J. Buerkler, D. Guerini, J. Brunner, and E. Carafoli.** 1984. 3-(Trifluoromethyl)-3-(m-[^{125}I]iodophenyl) diazirine, a hydrophobic, photoreactive probe, labels calmodulin and calmodulin fragments in a Ca^{2+}-dependent way. Biochemistry **23**: 400–403.
10. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
11. **Lodish, H. F., and M. Porter.** 1980. Heterogeneity of vesicular stomatitis virus particles: implications for virion assembly. J. Virol. **33**:52–58.
12. **Mancarella, D., and J. Lenard.** 1981. Interactions of wild type and mutant M protein of vesicular stomatitis virus with viral nucleocapsid and envelope in intact virions. Evidence from ^{125}I-iodonaphthylazide labeling and specific cross-linking. Biochemistry **20**:6872–6877.
13. **McSharry, J. J., and R. R. Wagner.** 1971. Lipid composition of vesicular stomatitis viruses. J. Virol. **7**:59–70.
14. **Miller, D. K., and J. Lenard.** 1980. Inhibition of vesicular stomatitis virus infection by spike glycoprotein. Evidence for an intracellular G protein-requiring step. J. Cell Biol. **84**:430–437.
15. **Morita, K., R. Vanderoef, and J. Lenard.** 1987. Phenotypic revertants of temperature-sensitive M protein mutants of vesicular stomatitis virus: sequence analysis and functional characterization. J. Virol. **61**:256–263.
16. **Morrison, T. G., and C. O. McQuain.** 1978. Assembly of viral membranes: nature of the association of vesicular stomatitis virus proteins to membranes. J. Virol. **26**:115–125.
17. **Mudd, J. A.** 1973. Effects of pH on the structure of vesicular stomatitis virus. Virology **55**:546–549.
18. **Odenwald, W. F., H. Arnheiter, M. Dubois-Dalcq, and R. A. Lazzarini.** 1986. Stereo images of vesicular stomatitis virus assembly. J. Virol. **57**:922–932.
19. **Ogden, J. R., R. Pal, and R. R. Wagner.** 1986. Mapping regions of the matrix protein of vesicular stomatitis virus which bind to ribonucleocapsids, liposomes, and monoclonal antibodies. J. Virol. **58**:860–868.
20. **Ono, K., M. E. Dubois-Dalcq, M. Schubert, and R. A. Lazzarini.** 1987. A mutated membrane protein of vesicular stomatitis virus has an abnormal distribution within the infected cell and causes defective budding. J. Virol. **61**:1332–1341.
21. **Pal, R., B. W. Grinnell, R. M. Snyder, J. R. Wiener, W. A. Volk, and R. R. Wagner.** 1985. Monoclonal antibodies to the M protein of vesicular stomatitis virus (Indiana serotype) and to a cDNA M gene expression product. J. Virol. **55**:298–306.
22. **Pepinsky, R. B., and V. M. Vogt.** 1979. Identification of retrovirus matrix proteins by lipid-protein cross-linking. J. Mol. Biol. **131**:819–837.
23. **Pringle, C. R.** 1975. Conditional lethal mutants of vesicular stomatitis virus. Curr. Top. Microbiol. Immunol. **69**:85–116.
24. **Reidler, J. A., P. M. Keller, E. Elson, and J. Lenard.** 1981. A fluorescence photobleaching study of VSV-infected BHK cells. Modulation of G protein mobility by M protein. Biochemistry **20**:1345–1349.
25. **Rose, J. K., and C. J. Gallione.** 1981. Nucleotide sequences of the mRNA's encoding the vesicular stomatitis virus G and M proteins determined from cDNA clones containing the complete coding regions. J. Virol. **39**:519–528.
26. **Rose, K., H. P. Kocher, B. M. Blumberg, and D. Kolakofsky.** 1984. An improved procedure, involving mass spectrometry, for N-terminal amino acid sequence determination of proteins which are Nα-blocked. Biochem. J. **217**:253–257.
27. **Schmidt, M. F. G., and M. J. Schlesinger.** 1979. Fatty acid binding to vesicular stomatitis virus glycoprotein: a new type of post-translational modification of the viral glycoprotein. Cell **17**:813–819.
28. **Sonderegger, P., R. Jaussi, H. Gehring, K. Brunschweiler, and P. Christen.** 1982. Peptide mapping of protein bands from polyacrylamide gel electrophoresis by chemical cleavage in gel pieces and re-electrophoresis. Anal. Biochem. **122**:298–301.
29. **Stoffel, W., C. Schreiber, and H. Scheefers.** 1978. Lipids with photosensitive groups as chemical probes for the structural analysis of biological membranes. On the localization of the G- and M-protein of vesicular stomatitis virus. Hoppe-Seyler's Z. Physiol. Chem. **359**:923–928.
30. **Wiener, J. R., R. Pal, Y. Barenholz, and R. R. Wagner.** 1983. Influence of the peripheral matrix protein of vesicular stomatitis virus on the membrane dynamics of mixed phospholipid vesicles: fluorescence studies. Biochemistry **22**:2162–2170.
31. **Wiener, J. R., R. Pal, Y. Barenholz, and R. R. Wagner.** 1985. Effect of the vesicular stomatitis matrix protein on lateral organization of lipid bilayers containing phosphatidylglycerol: use of fluorescent phospholipid analogs. Biochemistry **24**:7651–7658.
32. **Wilson, T., and J. Lenard.** 1981. Interaction of wild type and mutant M protein of vesicular stomatitis virus with nucleocapsids *in vitro*. Biochemistry **20**:1349–1354.
33. **Zakowski, J. J., W. A. Petri, Jr., and R. R. Wagner.** 1981. Role of matrix protein in assembling the membrane of vesicular stomatitis virus: reconstitution of matrix protein with negatively charged phospholipid vesicles. Biochemistry **20**:3902–3907.
34. **Zakowski, J. J., and R. R. Wagner.** 1980. Localization of membrane-associated proteins in vesicular stomatitis virus by use of hydrophobic membrane probes and cross-linking reagents. J. Virol. **36**:93–102.