the case of the glycoprotein, where it often appears to be associated with the B_1 transition. In fact, with the addition of 0.15 M NaCl at various pH's, the denaturation temperature of G is markedly reduced and occurs close to the A_1 transition. The response of G denaturation to pH and salt might be explained by proposing multiple structural domains for G which contribute to the A_1 as well as B_1 or B transitions.

Isolated components have been studied as well. In particular, the nucleocapsid exhibits one thermal transition at 73°C which involves the denaturation of N as shown by thermal gel analysis.

The effect of thermal treatment on the morphology of the virion was studied by negative stain electron microscopy. The results of these "Thermal EM" studies suggest that discrete morphological changes in the virion occur coincident with the three major calorimetric transitions.

This work was aided by grants S07-RR05349 and T32 AI-07150 from the National Institutes of Health.

Received for publication 1 May 1981.

REFERENCES

1. Eger, R., R. W. Compans, and D. B. Rifkin. 1975. The organization of the proteins of vesicular stomatitis virions: labeling with pyridoxal phosphate. *Virology.* 66:610–615.
2. Weiss, R. A., and P. L. P. Bennett. 1980. Assembly of membrane glycoproteins studied by phenotypic mixing between mutants of vesicular stomatitis virus and retroviruses. *Virology.* 100:252–274.
3. Mudd, J. A. 1974. Glycoprotein fragment associated with vesicular stomatitis virus after proteolytic digestion. *Virology.* 62:573–577.
4. Dubovi, E. J., and R. R. Wagner. 1977. Spatial relationships of the proteins of vesicular stomatitis virus: induction of reversible oligomers by cleavable protein cross-linkers and oxidation. *J. Virol.* 22:500–509.
5. Rose, J. K., W. J. Welch, B. M. Sefton, F. S. Esch, and N. C. Ling. 1980. Vesicular stomatitis virus glycoprotein is anchored in the viral membrane by a hydrophobic domain near the COOH terminal. *Proc. Natl. Acad. Sci. U.S.A.* 77:3884–3888.
6. Lysko, K. A., R. Carlson, J. Snow, and J. F. Brandts. 1981. *Biochemistry.* In press.

THE M PROTEIN OF VESICULAR STOMATITIS VIRUS

VARIABILITY IN LIPID-PROTEIN INTERACTION COMPATIBLE WITH FUNCTION

JOHN LENARD, DENISE A. MANCARELLA, TAZEWELL WILSON, JEFFREY A. REIDLER, PAUL M. KELLER AND ELLIOT L. ELSON

Department of Physiology and Biophysics, College of Medicine and Dentistry of New Jersey, Rutgers Medical School, Piscataway, New Jersey 08854, U.S.A.

Vesicular stomatitis virus (VSV) is a simple enveloped virus readily grown in continuous cultures of baby hamster kidney (BHK) cells and other cell lines. The major structural proteins of the virion are three virus-coded polypeptides, which together account for > 90% of the viral protein. The viral envelope consists of a bilayer of lipids derived from the plasma membrane of the infected host cell during budding, and a single glycoprotein, G, mol wt ~ 65,000 (1). A second major viral protein, the matrix or M protein, is a nonglycosylated polypeptide, mol wt 27,000. The N protein (mol wt ~ 50,000) associates with the RNA genome to form the viral nucleocapsid.

Recent experiments utilizing VSV temperature-sensitive (ts) mutants and viral pseudotypes have shown that M protein is essential for the budding of virions or virus-like particles from the plasma membrane of an infected cell (2–4). Specific functional interactions between M protein and viral nucleocapsids, and between M protein and patches of membrane containing G protein, are implied by these findings.

This paper summarizes experiments in which wild-type (wt) VSV and ts mutants with lesions in the M protein (ts M) were compared under conditions where the mutant M protein was functional; the differences found reflect permissible alterations that maintain M protein function. Four different ts M mutants were routinely studied and all gave qualitatively the same results in all the experiments described; for brevity, results for a single mutant are reported here. Temperature stable revertants were found to revert to wt behavior, indicating that the altered property of the ts M mutant was related to its phenotype. These experiments are reported in detail elsewhere[1] (5, 6).

RESULTS

M Protein-Membrane Interactions

Labeling of Intact Virions with ^{125}I-Iodoaphthylazide (INA). The hydrophobic photoreactive probe INA partitions preferentially into hydrophobic regions, most notably the interior of lipid bilayers (7, 8). It has been successfully used as a probe for membrane-associated proteins (9–12). We have found that the M protein in ts M

[1]Mancarella, D. A., and J. Lenard. 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 labelling and specific cross-linking. *Biochemistry.* In press.

BIOPHYS. J. © Biophysical Society · 0006-3495/82/01/26/03 $1.00
Volume 37 January 1982 26–28

TABLE I
LABELING WITH ^{125}I-INA*

	N	M
wt VSV	0.12	0.37
ts 33	0.16	0.74
ts 33r5	0.21	0.47

*Expressed as ^{125}I-INA/protein, normalized to G ± 1.0.[1]

virions is labeled two to three times as much as the M protein in wt virions. Temperature-stable revertants exhibit wt behavior[1] (Table I).

Fluorescence Photobleaching Studies of Cells Infected with WT and TS M VSV. These experiments measured the mobility of newly synthesized G protein on the surface of cells infected with either wt or ts M VSV. Fluorescein-labeled Fab fragments of anti-G antibody were used to visualize surface G protein. The mobile fraction of G protein on the surface of ts M infected cells was substantially smaller than this fraction on the surface of wt-infected cells at the permissive temperature (Table II). The rate of viral budding also decreased in ts M-infected cells, suggesting that the coordinate changes in affinities of the mutant M protein give rise to a different rate-limiting step for budding. The very large mobile fraction on wt-infected cells suggests that most of the surface G protein is not associated with M at any particular time; much more of the surface G appears to be M associated, and hence immobile, in ts M infected cells (5).

M Protein-Nucleocapsid Interactions

Abundance of a Disulfide-Linked M-N Complex Present after Nonreducing Disruption of WT and TS M Virions. The abundance of disulfide cross-linked M–N complex present after disruption of virions with sodium dodecyl sulfate in the absence of thiols was measured after separation on polyacrylamide gels (13). The amount of this complex was substantially reduced with ts M mutants as compared with either wt or revertants (Table III). This suggests a decreased association between M protein and nucleocapsids in intact ts M virions (7).

TABLE II
MOBILE FRACTION OF *G* PROTEIN ON THE SURFACE OF INFECTED CELLS

Infecting virus	Mobile fraction	
	31°	39°
	(%)	
wt VSV	74 ± 13 (19)*	73 ± 13 (52)
ts 33	48 ± 13 (18)	75 ± 12 (5)

*Number of measurements in parentheses (reference 5).

TABLE III
M-N COMPLEXES AFTER NON-REDUCTIVE DISSOCIATION IN SDS

	M-N/G*
wt VSV	0.41
ts 33	0.18
ts 33r5	0.44

*Average values from three experiments.

M-Nucleocapsid Affinity After Disruption with Nonionic Detergents. M protein is an effective inhibitor of the RNA polymerase activity of the nucleocapsid (mol) of VSV (14). In wt VSV or in ts M revertant preparations, much higher concentrations of salt are required to reverse this inhibition, i.e., to disrupt the M-nucleocapsid interactions, than in ts M preparations (Fig. 1). This indicates that M protein has predominantly electrostatic interactions with functional nucleocapsids; these interactions are apparently weakened by mutation to a ts M form (6).

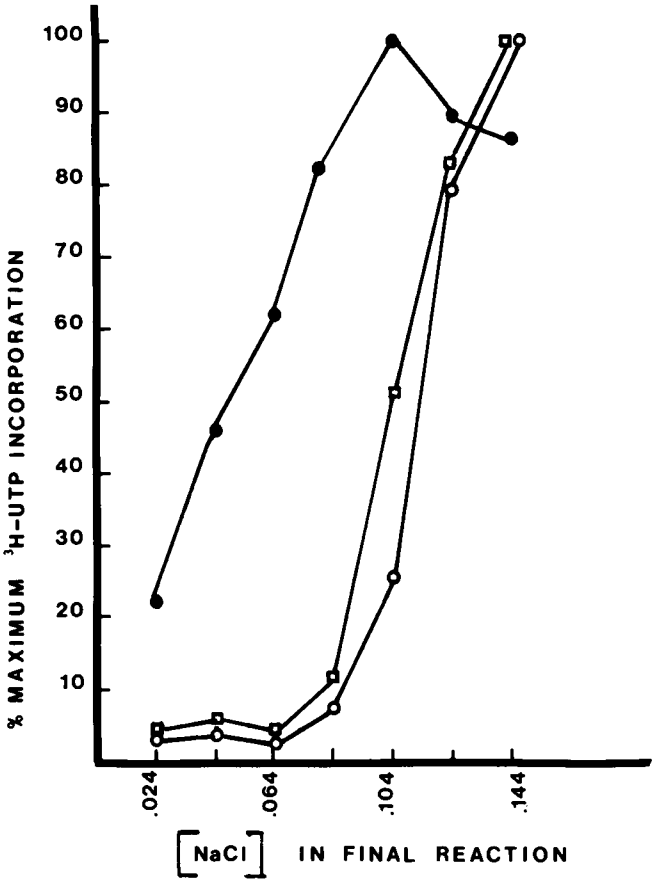

FIGURE 1 M protein-mediated inhibition of VSV transcription in vitro. The NaCl-dependence of in vitro transcription, measured by incorporation of H-UTP, was tested, Wt VSV (□—□) and 33r5 (○—○), at tsM revertant, exhibited similar patterns of NaCl-dependence in the reaction. M protein-mediated inhibition was reversed at lower NaCl concentrations when ts33 (●—●), at tsM mutant, was tested.

DISCUSSION

These results yielded the following conclusion: all ts M mutants exhibit a coordinate increase in association of M with membrane (or viral envelope) and a decrease in association with nucleocapsid. The mechanism for this alteration in phenotype cannot be fully understood until the complete structures of the wt and ts M protein are known. However, one can construct a simple explanation for the coordinate alterations by assuming that two independent binding sites govern the affinity of M for membranes and nucleocapsids, and that the mutation directly affects only the nucleocapsid binding site. The coordinate effect could then be produced simply by mass action:

$$\text{M - Nucleocapsid} \rightleftharpoons \text{M}_{\text{sol}} \rightleftharpoons \text{M - Membrane}.$$

Alternatively, there could be a portion of the M polypeptide that interacts with the membrane only under conditions of reduced interaction with the nucleocapsid. In this case, the two binding sites would not be completely independent.

Results with the ts M mutants demonstrate that the biological function of the M protein can be maintained in at least two different conditions of lipid-protein association; whether these two conditions are statistical or conformational is not addressed by our findings.

Received for publication 1 May 1981.

REFERENCES

1. Bishop, D. H. L., and M. S. Smith. 1977. Rhabdoviruses. *In* The Molecular Biology of Animal Viruses. D. P. Nayak, editor. Marcel Dekker, Inc., New York. 167–280.
2. Schnitzer, T. J., and H. F. Lodish. 1979. Non-infectious vesicular stomatitis virus particles deficient in the viral nucleocapsid. *J. Virol.* 29:443–447.
3. Schnitzer, T. J., C. Dickson, and R. A Weiss. 1979. Morphological and biochemical characterization of viral particles produced by the ts 045 mutant of vesicular stomatitis virus at restrictive temperature. *J. Virol.* 29:185–195.
4. Weiss, R. A., and P. L. P. Bennett. 1980. Assembly of membrane glycoproteins studied by phenotypic mixing between mutants of vesicular stomatitis virus and retroviruses. *Virology.* 100:252–274.
5. Reidler, J. A., P. M. Keller, E. L. Elson, and J. Lenard. 1981. A fluorescence photobleaching study of vesicular stomatitis virus infected BHK cells. Modulation of G protein mobility by M protein. *Biochemistry.* 20:1345–1349.
6. 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.
7. Sigrist-Nelson, K., H. Sigrist, T. Bercovici, and C. Gitler. 1977. Intrinsic proteins of the intestinal microvillus membrane. Iodonaphthylazide labeling studies. *Biochim. Biophys. Acta.* 468:163–176.
8. Bercovici, T., and C. Gitler. 1978. 5-(^{125}I)iodonaphthylazide, a reagent to determine the penetration of proteins into the lipid bilayer of biological membranes. *Biochemistry.* 17:1484–1489.
9. Kahane, I., and C. Gitler. 1978. Red cell membrane glycophorin labeling from within the lipid bilayer. *Science (Wash., D.C.).* 201:351–352.
10. Karlish, S. J. D., P. L. Jorgensen, and C. Gitler. 1977. Identification of a membrane-embedded segment of the large polypeptide chain of (Na^+, K^+) ATPase. *Nature (Lond.).* 269:715–717.
11. Cerletti, N. and G. Schatz. 1979. Cytochrome oxidase from bakers' yeast; photolabeling of subunits exposed to the lipid bilayer. *J. Biol. Chem.* 254:7746–7751.
12. 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.
13. Dubovi, E. J., and R. R. Wagner. 1977. Spatial relationships of the proteins of vesicular stomatitis virus: induction of reversible oligomers by cleavable protein cross-linkers and oxidation. *J. Virol.* 22:500–509.
14. Carroll, A. R., and R. R. Wagner. 1979. Role of the membrane (M) protein in endogenous inhibition of *in vitro* transcription by vesicular stomatitis virus. *J. Virol.* 29:134–142.

INSERTION OF BACTERIOPHAGE M13 COAT PROTEIN INTO MEMBRANES

MARCIA MANNING, SUSAN CHRYSOGELOS, AND JACK GRIFFITH

Cancer Research Center, Departments of Bacteriology and Immunology, Biochemistry and Nutrition, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27514 U.S.A.

The filamentous bacterial viruses (M13, fd, f1) provide an exceptionally good model system for studying factors that govern the insertion of proteins into membranes. 2,700 copies of the viral B protein are arranged with helical symmetry around the circular single-stranded DNA to form a long filamentous virion so tightly constructed that it is impervious to detergents, urea, proteases, and heating. However, during phage penetration, this tight association is somehow altered to allow insertion of the phage coat protein into the cell membrane and release of the viral DNA. In vitro studies have shown that isolated B protein can be incorporated into synthetic lipid vesicles as the vesicles are formed, and that maximum incorporation occurs at the phase transition temperature of the lipid (1). However, isolated B protein does not insert into preformed lipid bilayers. Apparently, in vivo, the construction of the virion facilitates insertion of the protein into the membrane, but this insertion mechanism has been difficult

BIOPHYS. J. © Biophysical Society · 0006–3495/82/01/28/03 $1.00
Volume 37 January 1982 28–30