STRUCTURAL DOMAINS IN VESICULAR STOMATITIS VIRUS MEMBRANE AS STUDIED BY DIFFERENTIAL SCANNING CALORIMETRY

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Vesicular stomatitis virus (VSV) is an enveloped RNA-containing virus which obtains its membrane lipid from the plasma membrane of infected cells. VSV contains three major structural proteins, two of which are membrane-associated: the matrix (M) protein lies against the inner surface of the membrane (1, 2) and the glycoprotein (G) extends completely through the membrane with the majority of the protein projecting through the external surface (3–5). The nucleocapsid (N) protein is complexed with the viral RNA and forms the helical core of the virion. High-sensitivity differential scanning calorimetry (DSC) has been used in combination with thermal gel analysis (6) to identify and study the structural domains in VSV. The identity of the components of each structural domain and possible interactions between them may be useful in determining the mechanism of viral assembly within the host cell.

DSC spectra of intact virus are obtained with a Microcal MC-1 differential scanning calorimeter (Microcal; Amherst, MA) as a function of pH (5.5–9.0) and salt (±0.15 M NaCl) in 0.01 M phosphate buffer. At pH 7.0 the total enthalpy change, Δ, between 45° and 83°C is estimated to be 4–8cal/g of protein. At least five endothermic transitions are observed (Fig. 1 *A*). The minor transitions A_1 and B_1 are not well-defined and occasionally are not apparent. Although the temperatures at which the *B* and *C* transitions occur are reproducible, the resolution between them varies between preparations of virus for unknown reasons. The *A* transition appears to be reversible although it is shifted to lower temperatures (43° vs. 53°C) upon subsequent scans. Based partly on thermal gel analysis it is speculated that the second transition seen at 43°C, after the sample has already been heated to 60°C once, arises from reversible unfolding of a multimer (tetramer?) of the M protein. After the virion is heated through the *B* and *C* transitions, all transitions become irreversible. Increasing pH affects the *B* and *C* transitions in a parallel manner as they gradually shift to lower temperatures. This parallel effect may reflect a structural link between the two.

A new analytical technique termed thermal gel analysis has been developed by Lysko et al. (6) to identify the proteins involved in the calorimetric transitions observed for the erythrocyte membrane. Using this method the midpoint denaturation of the viral proteins M, G and N (at pH 7.0) occurs coincident with the major calorimetric transitions *A*, *B*, and *C*, respectively (Fig. 1 *B*). As pH is varied, the denaturation of these proteins is seen to occur very near their respective calorimetric transitions except in

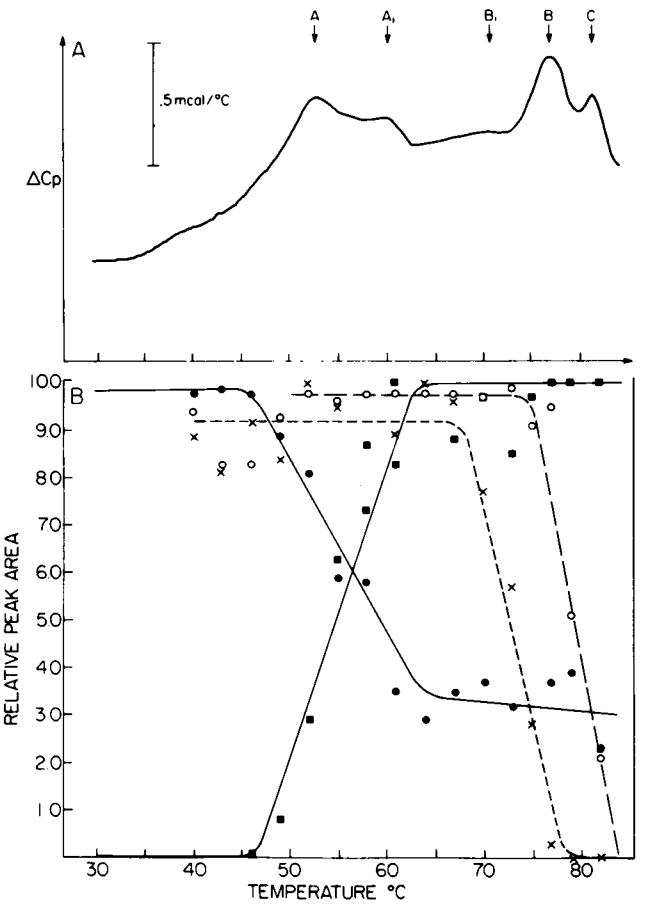

FIGURE 1 *A*, Heat capacity change as a function of temperature for intact VSV suspended in 0.01 M phosphate buffer, pH 7.0, at 1.4 mg/ml protein. Maximum point of each thermal transition labeled as *A*, 53°; A_1, 60°; B_1, 70°; *B*, 77°; *C*, 81°C. *B*, Normalized relative peak areas for major VSV proteins plotted as a function of the maximum temperature to which the virus was heated in 0.01 M phosphate buffer, pH 7.0. M (● — ●), M multimer (■ — ■), G (X- - -X), N (O —— O).

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.

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THE M PROTEIN OF VESICULAR STOMATITIS VIRUS

VARIABILITY IN LIPID-PROTEIN INTERACTION COMPATIBLE WITH FUNCTION

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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