Maturation Defect of a Temperature-Sensitive Mutant of Western Equine Encephalitis Virus

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With 6 Figures

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Summary

The defective step of a temperature-sensitive mutant of western equine encephalitis virus, which synthesize viral RNA but not mature virus at the restrictive temperature, was studied. Cells infected with the mutant virus at the restrictive temperature synthesized the same intracellular viral RNA as that in wild type infection. Cells infected with the mutant at the restrictive temperature formed three proteins (E1, E2 and C) which migrated to positions identical with those of purified virions and a precursor protein of E2 (PE2). The mutant virus was also able to form cytoplasmic nucleocapsids sedimenting at 140S as in the case of wild type infection. On the other hand, cells infected with the mutant could not induce a significant amount of hemadsorbing ability and the ability induced at the permissive temperature disappeared immediately after shifting up to the restrictive temperature. These results suggested that the mutant virus produced a defective envelope protein responsible for hemagglutination at the restrictive temperature. Owing to the incompleteness of the modification of the cell plasma membrane by the envelope proteins, viral nucleocapsids in the mutant infected cells could not bind to the plasma membrane.

Introduction

Alphaviruses are composed of a spherical nucleocapsid surrounded by an envelope consisting of lipids and viral glycoproteins (13). Because of their relative chemical simplicity in enveloped viruses, alphaviruses have been employed extensively as a model system to study viral envelope acquisition. Morphogenesis of alphaviruses is divided into the following steps: 1. formation of nucleocapsid from the capsid protein and the virion RNA, 2. modification of a cell plasma membrane by the addition of envelope protein E1 and E2 with the exclusion of
the cellular proteins, 3. transportation of the nucleocapsid to specific areas of the cell plasma membrane by some process not yet determined, 4. budding of the nucleocapsid through the modified plasma membrane to release mature virions into the extracellular fluid (13).

Temperature-sensitive (ts) mutants of Sindbis (SIN) or Semliki Forest (SF) virus have been used for the study of the process of virus multiplication (1, 8, 10, 11, 13, 17). We have previously reported the selection and preliminary characterization of a ts mutant of western equine encephalitis (WEE) virus closely related to SIN or SF virus (16). The ts mutant did not produce infectious virus at a restrictive temperature but it was able to stimulate substantial viral RNA synthesis (RNA+ mutant). The mutant was also shown to be heat labile at an elevated temperature, indicating a structural protein defect. Therefore, the ts mutant may offer an opportunity to study the process of virus maturation. We report here that the mutant may be defective in one of the envelope proteins possessing hemagglutinating activity and discuss the process of virus maturation.

Materials and Methods

Cell Culture and Virus

Primary chick embryo (CE) cell cultures were prepared by the same method as described in a previous paper (15). Virus stocks were made from CE culture fluid infected with wild type (WT) and a ts mutant (ts-39) of WEE virus. The isolation and preliminary characterization of ts-39 strain have been reported (16). Monolayers of CE cells in culture bottles were treated with 1 μg of actinomycin-D (Act-D) per ml for an hour, and infected with WT or ts-39 virus at an input multiplicity of 10 PFU per cell. After a 45-minute adsorption at 37°C, the infected cultures were incubated at 37°C (permissive temperature) or 41.5°C (restrictive temperature). Incubation at 41.5°C was done in a water-bath controlled within ±0.1°C.

Analysis of Viral RNA and Nucleocapsid

Velocity sedimentation analyses of viral RNA and nucleocapsid were performed as described elsewhere (7). Infected cells were incubated at 41.5°C and labeled with ³H-uridine (10 μCi/ml) for 2 hours at 2 hours postinfection (p.i.). At the end of the labeling period, viral RNA was extracted with 1 per cent SDS for RNA analysis or the cell cytoplasm was prepared by 0.5 per cent Nonidet P-40 (NP-40) for nucleocapsid analysis from the infected cells.

Gel Electrophoretic Analysis of Viral Protein

Act-D-treated cell cultures were infected with WT or ts-39. After virus adsorption, 2 ml of Eagle’s medium (MEM) containing one-tenth of the standard amount of amino acids was added to each culture, which was added with ¹⁴C-protein hydrolysate (2 μCi/ml in final) at 3 hours p.i. and incubated for 2 hours. The cytoplasmic extract was prepared with 0.5 per cent NP-40, treated with 1 per cent SDS, 1 per cent 2-mercaptoethanol and heated at 100°C for 2 minutes. The discontinuous SDS-gel electrophoresis was performed according to the method described by LAEMMLI (12) with a slight modification (7).

Hemadsorption Test

Hemadsorption test was carried out essentially according to the method described by BURGE and PFEFFERKORN (3). Eight percent suspension of goose red cells in barbiturate buffer containing glucose and gelatin (4) was washed once with the same buffer, twice with 1:1 mixture of the borate and pyrophosphate buffers (pH 6.0 in final) used in hemagglutination titration (4). The red cells were resuspended at a 3 per cent concentration in the borate-pyrophosphate buffers for hemadsorption test.