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STRUCTURAL PROTEINS OF GETAH VIRUS ISOLATES FROM JAPAN AND MALAYSIA

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Summary. — Purified preparations of Getah virus strains have been analysed by sodium-dodecyl-sulphate polyacrylamide gel electrophoresis (SDS-PAGE) to reveal their structural proteins. Two envelope proteins (E1 and E2) and core protein (C) were found with the prototype AMM2021 strain both under reducing and nonreducing conditions, while separation of E1 and E2 was observed only under nonreducing conditions for 3 strains isolated in Japan. Limited digestion by *Staphylococcus aureus* V8 protease revealed difference in the peptide patterns of E1 between AMM2021 and Japanese isolates. Mobility of E1 and E2 was slower for the virus grown in BHK21 cells compared with the virus grown in *Aedes albopictus* cells, indicating host-controlled modification on the envelope glycoproteins.

Key words: Getah virus, structural protein, strain difference

Introduction

Getah virus is an alphavirus (family Togaviridae), characterized by a cubic nucleocapsid surrounded by envelope with glycoprotein projections, as reviewed for Sindbis or Semliki Forest (SF) viruses (Pfefferkorn and Shapiro, 1974). The nucleocaspid contains single-stranded RNA of plus polarity and core protein, while the envelope consists of host derived lipid bilayer and virus-specified glycoproteins, E1 and E2 (and in the case of SF virus also E3).

The prototype of Getah virus had been isolated in Malaysia in 1955 (Elisberg and Buescher, 1963), and the Sagiyama strain isolated in Japan in the following year was found to be closely related to the former (Scherer *et al.* 1962). Virus isolation and serology have shown that Getah virus is widely distributed in Japan and Southeast Asia to Australia (Doherty *et al.* 1963, Matsuyama *et al.* 1968, Tesh *et al.* 1975, Simpson *et al.* 1975, Marchette *et al.* 1978; 1980, Yamamoto 1980, Sentsui and Kono, 1980, Igarashi *et al.* 1981*a*; *b*; *c*). Although the pathogenicity for humans remains uncertain, recent outbreak of febrile disease among race horses with eruption and respiratory symptoms was caused by Getah virus infections (Sentui and Kono, 1980, Wada *et al.* 1982). Several reports indicated strain differences

of Getah virus either by antigenic analysis (Berge 1975, Chanas *et al.* 1976, Kimura and Ueba, 1978; Kamada *et al.* 1980; 1982) or by oligonucleotide fingerprint analysis (Morita and Igarashi, 1984). However, structural proteins of Getah virus have not been well characterized, nor the strain differences in terms of peptide analysis. This report describes the first characterization of the structural proteins of Getah virus in relation to their strain differences as revealed by SDS-PAGE.

Materials and Methods

Cells. Aedes albopictus, clone C6/36, cells were grown at 28 °C with cell growth medium of Eagle's minimal essential medium supplemented with 0.2 mmol/l each of nonessential amino acids (Eagle, 1959) and 10% heat-inactivated foetal calf serum (Igarashi 1978). BHK21 cells were grown at 37 °C with the same medium.

Virus. Prototype Getah virus, AMM2021, and 3 Japanese isolates, Sagiyama, B42, and P14, were used in the study. The origins of these strains were described previously (Morita and Igarashi 1984). The seed virus was prepared for each strain by growing in C6/36 cells to high titer exceeding 10^8 PFU/ml.

Purification of the virus. Confluent cultures of C6/36 or BHK21 cells in Roux bottles were inoculated with 1 : 100 diluted seed virus using 2 ml/bottle. After 2 hr adsorption, the cells were covered by 40 ml/bottle of the maintenance medium (the cell growth medium from which serum concentration was reduced to 2%) and incubated at 28 °C for C6/36, or at 37 °C for BHK21 cells. Infected culture fluid was harvested between 24 to 48 hr after infection using the cytopathic effect as indicator. The fluid (250 to 500 ml volume) was centrifuged at 2500 rev/min for 15 min, and the supernatant was added with polyethylene glycol 6000 and NaCl to 6 % and 0.5 mol/l, respectively. The mixture was centrifuged at 10,000×g for 30 min, and the precipitate was dissolved in 6 ml of STE buffer (0.1 mol/l NaCl, 0.01 mol/l Tris-HCl, 0.001 mol/l EDTA, pH 7.4) and centrifuged at 2500 rev/min for 15 min. The supernatant was layered on top of 2 ml of 15 % (w/w) sucrose column and 4 ml of 30—50 % (w/w) linear sucrose gradient in STE buffer and centrifuged at 25,000 rev/min for 12 hr at 4 °C in an SW41 rotor of Beckman ultracentrifuge. Fractions of 0.4 ml vol were collected by ISCO density gradient fractionator, model 640, and the peak fractions of OD_{254} were used as purified virus specimens.

SDS-PAGE. Slab gel method (Studier, 1973) with discontinuous buffer system (Laemmli 1970) was used with 10 % gel (acrylamide-bisacrylamide ratio of 30 : 0.8) in 1 mm thickness. Specimens were solubilized either under reducing condition (1 % SDS and 1 % 2-mercaptoethanol) or under nonreducing condition (1 % SDS and 0.5 mg/ml of iodoacetoamide) in 0.125 mol/l Tris-HCl, pH 6.8, by heating at 100 °C for 1 min before electrophoresis (Durbin and Stollar, 1984). Protein bands were visualized by staining with 0.1 % Coomassie Brilliant Blue R250 in 10 % acetic acid and 30 % methanol followed by destaining with 10 % acetic acid and 30 % methanol by diffusion.

V8 protease digestion and peptide mapping. The procedure was as described by Cleveland *et al.* (1977). Protein band in 1 mm gel was cut out and digested with *Staphylococcus aureus* V8 protease in the slot of 1.5 mm gel in the presence of 0.1 % SDS followed by the electrophoresis and staining.

Chemicals. Iodoacetoamide, acrylamide, bis acrylamide, and 2-mercaptoethanol were the products of Wako Pure Chemicals Co. V8 protease was obtained from Sigma Chemicals, USA.

Results

Structural proteins of Getah virus strains

The prototype Malaysian isolate, AMM2021, and 3 Japanese strains, Sagiyama, B42, and P14, grown in C6/36 cells and B42 strain grown in BHK21 cells were analysed for their structural proteins by SDS-PAGE under reducing as well as nonreducing conditions and the results were shown in Fig. 1. Three bands of structural proteins were revealed for

AMM2021 both under reducing and nonreducing conditions. Preliminary studies have shown that E1 and E2 were solubilized by nonionic detergent while C remained with RNA to form nucleoprotein core. Estimated molecular weights of E1, E2, and C proteins were 48K, 47K, and 33K daltons, respectively. Two envelope proteins, E1 and E2, were well-separated only under nonreducing conditions for 3 Japanese strains. Mobilities of these envelope proteins were slower for the B42 strain grown in BHK21 cells compared with the same strain grown in C6/36 cells. The result showed that the envelope proteins of a Malaysian strain were apparently different from those of Japanese strains, and no significant difference was observed between the structural proteins of old (Sagiyama) and new (B42) Japanese isolates, or between the P14 host-dependent temperature-sensitive mutant (Igarashi *el al.* 1981*d*) and its parental strain (B42).

Peptide mapping by V8 protease digestion

Structural proteins of AMM2021, and 3 Japanese strains grown in C6/36 cells were separated by SDS-PAGE and each band was cut out and digested by V8 protease followed by gel electrophoresis and the results were shown in Fig. 2. Difference was observed between the E1 peptide patterns of AMM2021 and other strains, however, no significant difference was observed for those of E2 and C proteins.

Discussion

Morita and Igarashi (1984) showed that AMM2021 is remote from Japanese strains of Getah virus by RNA oligonucleotide fingerprint analysis, which is compatible with the present results. However, no significant difference was observed between an old Sagiyama and new B42 Japanese isolates or between a host-dependent temperature-sensitive mutant, P14, and its parental strain, B42, by the present study on their structural proteins, although oligonucleotide fingerprint showed distinct differences (Morita and Igarashi 1984). Therefore, it appears that the peptide analysis is less sensitive than RNA fingerprint to reveral strain differences, or the difference in the RNA fingerprint could mostly represent the difference in the coding region for nonstructural proteins.

In our study on the structural proteins of Getah virus by protein staining, it would be absolutely necessary to use purified virus preparations which is free from contaminating non-viral proteins. In our virus purification procedures, we were very much careful about this point and final preparation appeared to be consisting mostly of viral structural proteins, although some faint accessory bands were stained in the SDS-PAGE (Fig. 1). Better separation of envelope proteins, E1 and E2, was obtained under nonreducing conditions using iodoacetamide compared with reducing conditions using 2-mercaptoethanol as reported for Sindbis virus (Durbin and Stollar, 1984), especially for Japanese isolates. This is another indication that the prototype Malaysian AMM2021 strain is significantly different from other Japanese strains in terms of their envelope proteins, although

the difference was not well reflected in the antigenic differences (Kamada *et al.* 1982). Peptide mapping indicated that the difference was more marked in E1 than in E2. Different mobilities of envelope proteins of B42 strain grown in BHK21 cells and in C6/36 cells could well be explained by the difference in the glycosyl residues on the envelopes because it is known that Sindbis virus grown in mosquito cells did not contain sialic acid and the glycosylation of envelope protein is controlled by the host enzyme (Stollar *et al.* 1976).

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Explanation to Figures (Plates XIII—XIV).

Fig. 1. Separation of structural proteins of Getah virus strains by SDS-PAGE under reducing and non-reducing conditions. Purified preparations of Getah virus strains were analysed by 10% SDS-PAGE as documented in the Materials and Methods, under reducing (1—6) or non-reducing (7—12) conditions. Specimens were: 1 and 7, molecular weight marker; 2 and 8, AMM2021 grown in C6/36; 3 and 9, Sagiyama grown in C6/36; 4 and 10, P14 grown in C6/36; 5 and 11, B42 grown in C6/36; 6 and 12, B42 grown in BHK21.

Fig. 2. Peptide mapping of structural proteins of Getah virus strains by limited digestion with *Staphylococcus aureus* V8 protease followed by SDS-PAGE. Structural proteins of P14 (lanes 1, 5, 9); B42 (2, 6, 10); Sagiyama (3, 7, 11); and AMM2021 (4, 8, 12) were separated by the 10% SDS-gel and each band of E1 (1, 2, 3, 4) E2 (5, 6, 7, 8), and C (9, 10, 11, 12) was cut out and treated with VB protease 0.5 mg/ml, at room temperature for 30 min in the presence of 0.1% SDS in the stacking gel of 1.5 mm thickness.