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COMPARISON OF THE PROTEIN COMPOSITION OF TWO PLAQUE VARIANTS OF SENDAI VIRUS

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Summary. — The protein composition of plaque variants of Sendai virus were compared by slab polyacrylamide gel electrophoresis. The RL and RS variants displayed clearly different P-protein mobilities; the P protein of the RS variant migrated faster than that of the RL variant, while no difference was found with proteins L, HANA, NP, F_1 and M. These protein patterns differed from those of the Fushimi and Z strains.

Key words: Sendai virus; *Paramyxovirus*; *plaque variants*; *protein composition*

Introduction

The biological activities of large (RL) and small (RS) plaque variants of Sendai virus (genus *Paramyxovirus*) isolated in this laboratory from the same source (Sugita *et al.*, 1974), were compared. We found that the cleavage of the precursor F protein of the RL variant is more sensitive to trypsin treatment than that of the RS variant and that the haemagglutinating (HA) and neuraminidase (ND) activities of the RS variant are more thermostable than those of the RL variant (Sugita *et al.*, 1981).

The different biological activities of the two variants prompted a study as to whether differences exist in their protein composition. In addition, the protein compositions of the two variants were compared to those of two other strains (Fushimi and Z) of Sendai virus reported to possess different protein compositions (Etkind *et al.*, 1980).

Sendai virus grown in embryonated eggs has seven proteins; three of them are glycoproteins called HANA, F_1 and F_2, and the others are nonglycosylated proteins designated as L, P, NP, and M (Homma *et al.*, 1975). The largest protein, L, has been identified as a nucleocapsid component (Lamb *et al.*, 1979). The second largest protein, P, is a constituent of the transcriptase-active nucleocapsid (Marx *et al.*, 1974). The third largest protein, HANA, has both HA and ND activities (Tozawa *et al.*, 1973). F_1 protein carries both haemolytic and cell fusion activities (Homma and Ohuchi, 1973; Scheid and Chopin, 1974), and F_1 and F_2 proteins are the cleavage products of the precursor F proteins (Homma and Ohuchi, 1973). M protein is a matrix protein and plays an important role as a limiting factor in the assembly of viral components (Yoshida *et al.*, 1976).

Materials and Methods

The following four strains of Sendai virus were used. The RL and RS strains were isolated from a rat in our laboratory; for their characteristics see Sugita *et al.* (1981). The Fushimi strain (Kuryoa *et al.*, 1953) was kindly provided by Dr. N. Ishida, Tohoku University, and the Z strain (Fukai and Suzuki, 1955) by Dr. Y. Hosaka, Osaka University. These two strains had undergone numerous passages in embryonated eggs. The RS, Fushimi and Z viruses were grown in embryonated eggs for 3 days (the RL strain for 4 days) and all strains were partially purified by two cycles of differential centrifugation (5,000 × g for 15 min and 43, 000 × g for 30 min in a Hitachi RP30A rotor. Slab polyacrylamide gel electrophoresis (PAGE) was carried out in principle according to Laemmli (1970). To partially purified virions of each strain at a protein concentration of 1 mg/ml was added 1 % sodium dodecyl sulfate and 1 % β-mercaptoethanol and the mixture was heated at 100 °C for 2 min, and then supplied with 10 % sucrose and 0.005 % bromophenol blue as a marker dye. Acrylamide gels, 2 mm thick, were 12.5 % for the separating gel and 5 % for the stacking gel. Samples of 20 or 40 μl were placed on the gels. Electrophoresis was conducted for 16 hr at a constant current of 20 mA. The gels were stained in a mixture of 50 % methanol, 7 % acetic acid and 0.2 % Coomassie brilliant blue R-250 for 15 min, then destained by repeated washings in 10 % methanol and 7 % acetic acid, and dried under vacuum on to chromatographic paper.

Results and Discussion

The protein compositions of RL and RS viruses are compared in Fig. 1 They were characteristic of Sendai virus and the only difference detected was in the electrophoretic mobility of P protein. It may be concluded that the difference in P protein may be correlated with a recent finding that the onset of RNA synthesis is earlier for RS than for RL (Sugita, in preparation). However, no differences were found in the patterns of HANA and F_1 proteins between the two plaque variants, which does not reflect the biological differences in HA and ND activities between them.

Different protein patterns in SDS-PAGE were also found when the protein compositions of these two variants were compared with those od Fushimi and Z strains of Sendai virus which were recently reported to possess different protein compositions (Etkind *et al.*, 1980). These authors showed by slab PAGE that strain-specific differences existed in the mobility of the protein synthesized in chick embryo cells infected with various Sendai virus strains (MN, Z, Fushimi, Obayashi and RU) and that only Z differed from the other strains in protein composition; that is, it had a smaller NP protein and a larger M protein. The present study confirmed their result by comparison of the protein compositions of Fushimi and Z (Fig. 2, gels 1-3). On comparing the protein compositions of Z and RS (Fig. 2, gels 3-5), differences in the electrophoretic mobilities of P, NP, and M proteins were detected. HANA protein of RS seemed to run faster than that of Z but, as its mobility differed from experiment to experiment, I could not determine whether the mobility of the HANA proteins actually differs between these strains. In coelectrophoresis of RS and Fushimi, I found that RS had a smaller P protein and a larger NP protein (data not shown). Table 1 summarizes these results to give the approximate molecular weights of the proteins of each virus.

These results showed that RL and RS, both of the same origin, possessed similar protein patterns, except for the P protein. Fushimi and Z strains, which are of different origin, had protein patterns differing from those of RL

Table 1. Molecular weights of virion proteins of four strains of Sendai virus

Protein	Strain			
	RL	RS	Fushimi	Z
L	110,000	110,000	110,000	110,000
P	75,000	73,000	75,000	75,000
HANA	67,000	67,000	67,000	69,000
NP	61,000	61,000	60,000	59,000
F_1	51,000	51,000	51,000	51,000
M	35,000	36,000	35,000	37,000

These values were estimated according to the molecular weights of proteins of the Fushimi strain as data reported by Homma *et al.* (1975). F_2 protein could not be detected in the system employed here.

and RS. I have no comparative data on the biological activities of these four strains, except for the plaque morphology in $LLCMK_2$ cells. RL produced larger plaques than RS, and both types of plaques had the same jagged edges. Plaques of Fushimi had clear edges and were intermediate in size between those of RL and RS (Sugita *et al.*, 1974). Plaques of Z were similar to those of Fushimi.

The differences in the electrophoretic mobilities of P, NP, and M proteins of viral particles of the four strains of Sendai virus used here could also be recognized in the proteins synthesized in $LLCMK_2$ cells infected with each strain (Sugita, in preparation). An interesting phenomenon was observed concerning the interference in coinfection with two of these strains; the growth of RL was markedly restricted by coinfection with RS, whereas Fushimi and Z could grow well on coinfection with RS (Sugita, in preparation). The finding of different protein compositions provides an important clue to determine whether the interference occurs in the protein synthesis. To ascertain that the base sequence coding for these proteins (P, NP, and M) differ from one type of strain to another type, further analysis of the cleavage products of these proteins by proteolytic enzymes is now in progress.

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Explanations of Figures (Plates XLVI and XLVII):

Fig. 1. Comparison of protein compositions of two plaque variants of Sendai virus by SDS-PAGE. Gel 1 — RS variant; gel 2 — RS + RL variants; gel 3 — RL variant.

Fig. 2. Comparison of protein compositions of RS, Fushimi and Z strains of Sendai virus. Gel 1 — Fushimi strain; gel 2 — Fushimi + Z strain; gel 3 — Z strain; gel 4 — Z + RS strain; gel 5 — RS strain.

Viral proteins designated according to Homma *et al.* (1975).