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SHORT COMMUNICATION

ISOLATION OF THE M POLYPEPTIDE OF SENDAI VIRUS (HVJ) WITH COLUMN CHROMATOGRAPHY

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Fully infectious egg-grown Sendai virus (HVJ) consists of five major and some minor polypeptides (Shimizu et al., 1972; 1974; Homma and Ohuchi, 1973; Scheid and Choppin, 1974; Lam et al., 1976). The biological functions associated with these polypeptides have been identified or postulated (Content and Duesberg, 1970; Stone et al., 1972; Homma and Ohuchi, 1973; Tozawa et al., 1973; Hosaka et al., 1974; Marx et al., 1974; Shimizu et al., 1974; Shimizu and Ishida, 1975; Yoshida et al., 1976).

The M polypeptide is one of the non-glycosylated polypeptide in the major polypeptide constituents and probably takes a part for the matrix protein of the virion. Its biological role has recently been suggested by Shimizu and Ishida (1975) and Yoshida et al. (1976). The isolation of the M polypeptide in the paramyxovirus was carried out by Scheid et al. (1972, 1973) with the high salt-detergent method and by Yoshida et al. (1976) with the sarcosine-polyacrylamide gel electrophoresis method. However, the amino acid composition of the M polypeptide of Sendai virus has not yet been reported.

The present report describes a simple chromatographic method to isolate the M polypeptide and the result of amino acid analysis.

Sendai virus, Z strain, was used throughout the present investigation. The virus was inoculated into chorioallantoic cavities of 10-day-old embryonated eggs and incubated at 35C for 3 days. The virus produced in allantoic cavities was purified by two cycles of differential centrifugation at $3,300\times g$ for 15 min and $65,000\times g$ for 30 min and suspended in SSC (0.15 M sodium chloride and 0.015 M sodium citrate). The virus thus purified (4 ml, 40,000 HAU/ml) was pelleted at $65,000\times g$ for 30 min and the resulting pellet was suspended in 2 ml of 0.2 M sodium phosphate (pH 6.4) containing 1% sodium dodecyl sulfate (SDS, Nakarai Chemicals Co.), 1% 2-mercaptoethanol and 1 mM EDTA. This virus solution was heated at 100C for 10 sec to dissociate the virions. The resulting clear solution was chromatographed on a Bio-Gel P-200 column (100–200 mesh, Bio-Rad Lab., USA).

Figure 1 shows an elution profile of the SDS-solubilized virions on a Bio-Gel P-200 column. Three peaks, designated P1, P2 and P3, were separated. When the elution was run in a lower ionic strength (0.05 M sodium phosphate) in this Bio-Gel column chromatography, fraction P3 did not separate from fraction P2.

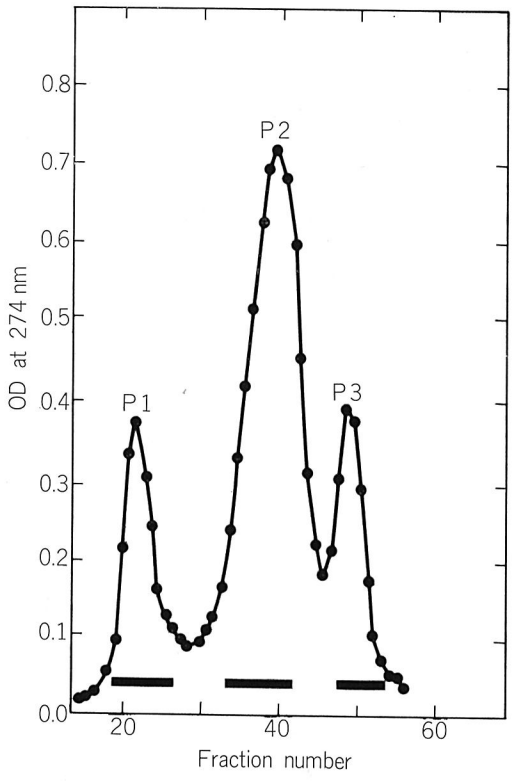

FIGURE 1. Bio-Gel P-200 chromatography of SDS-solubilized Sendai virus.
A Bio-Gel column (2×95 cm) previously equilibrated with 0.2 M sodium phosphate (pH 6.4) containing 0.1% SDS, 0.02 M 2-mercaptoethanol and 1 mM EDTA was loaded with SDS-solubilized virions (2 ml) and developed with the same solvent at a flow rate of 1.2 ml/hr at room temperature. Approximately 1.4 ml of the effluents were collected in a tube. Each fractions, P1, P2 and P3, indicated by bars were pooled and an aliquot of the pooled fraction was submitted to gel electrophoresis, respectively.

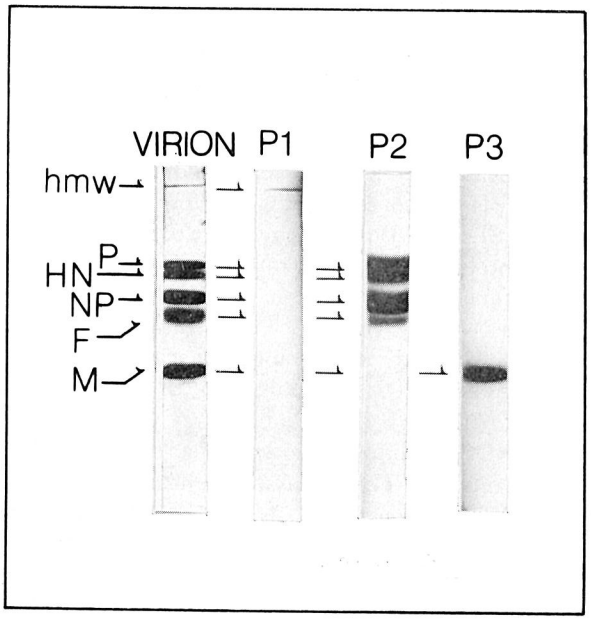

FIGURE 2. Gel electrophoresis pattern of Sendai virions, P1, P2 and P3.
SDS-electrophoresis was done in 7.5% polyacrylamide gels at 5 ma per gel for 6 hr and then the gels were stained by Coomassie brilliant blue.

In 0.01 M sodium phosphate, no viral polypeptide was eluted from the Bio-Gel column.

The SDS-gel electrophoretic behavior of the material in these three peaks are shown with that of the whole polypeptides of the virion (Fig. 2). A single polypeptide band corresponding to hmw was detected in fraction P1, which contained a lot of viral nucleic acid that absorbed maximally at 258 nm. The high content of the nucleic acid in this fraction was also shown in the lower absorbance at 280 nm than that at 260 nm (OD 280 nm/OD 260 nm = 0.62). This may be a reason why the hmw polypeptide was detected only as a faintly stained band, in spite of the moderate absorbancy at 274 nm which appeared in fraction P1. Four distinct bands were detected for polypeptides in fraction P2 on this electrophoresis. Based on the characteristic mobility of these four bands, it is deduced that four polypeptides, P, HN, NP and F, correspond to the individual bands. Fraction P3, the most retarded

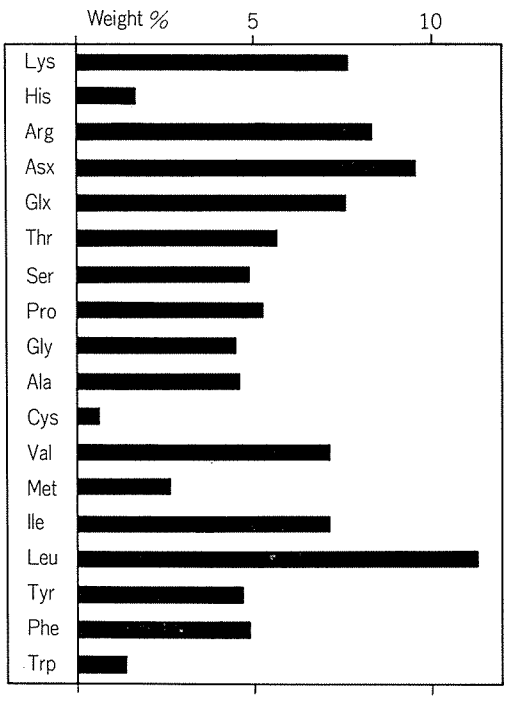

FIGURE 3. The amino acid composition of the M polypeptide of Sendai virus.
The amount of amino acids determined for the M polypeptide of Sendai virus is shown by bars. Hydrolysis of the M polypeptide of Sendai virus was carried out with 4 N methanesulfonic acid containing 0.2% tryptamine (Simpson et al., 1976).

fraction in the Bio-Gel P-200 chromatography, gave one band corresponding to the M polypeptide.

The pooled effluents in fraction P3 were first dialyzed for two days against 0.06 M sodium acetate containing 6 M urea at pH 5.2 with Dowex X 1 (acetate form), which was used to trap SDS. The urea solution was then completely dialized against distilled water by changing the water several times. Usually the M polypeptide precipitated nearly completely on first dialysis of the urea solution against distilled water. The precipitated M polypeptide was then collected by centrifugation as the moistened pellet, which was dried in vacuo over anhydrous calcium sulfate. The M polypeptide sample thus obtained was submitted to amino acid analysis.

The amino acid composition of the M polypeptide is shown in Fig. 3. Except for cystine, the values determined for the respective amino acid represent the average one obtained in duplicate determinations. Approximately half the amount of the M polypeptide sample was recovered as amino acid on the weight basis. A possible reason of the low recovery of amino acids is that the polypeptide sample may contain a considerable amount of SDS and water. Considering the isolation procedure used in the present work, it is unlikely to suppose that the polypeptide sample might be contaminated with other materials in addition to the above ones. Further, carbohydrate may not be contained in the polypeptide sample, since the M polypeptide is probably non-glycosylated.

It is interesting to note that the amino acid composition of the M polypeptide of Sendai virus is similar to that of the M polypeptide of paramyxovirus SV5 (McSharry et al., 1975).

The content of the hydrophobic amino acids is so high as to be added up to approximately 39 weight-% of the total amino acid composition, suggesting that the hydrophobicity of the M polypeptide may be closely related to the substantial affinity to the membrane lipid-bilayer (Shimizu and Ishida, 1975; Yoshida et al., 1976).

The N-terminal analysis of the M polypeptide was performed by dansylation in the presence of 2% SDS according to the procedure of Gray (1972). Identification of the dansylated amino acid was carried out by using thin-layer chromatography on polyamide sheets (5×5 cm) (Cheng Chin Trading Co., Taiwan) with acidic and basic solvent systems (Kimura, 1974).

In the chromatography using these two solvent systems, no N-α-dansyl amino acid was detected in the hydrolysate of the dansylated M polypeptide. Since it is difficult to suppose that the N-terminal group of the M poly-

peptide was resistant to the dansylation reaction under the present conditions, the present result of N-terminal analysis leads to the conclusion that the N-terminal residue in the polypeptide may be blocked by a certain substituent. However, at present a possibility that the acid-labile residue is present at the N-terminus is not excluded.

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