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Citation	Biken journal : journal of Research Institute for Microbial Diseases. 1979, 22(2), p. 71-74
Version Type	VoR
URL	https://doi.org/10.18910/82554
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SHORT COMMUNICATION

ISOLATION AND AMINO ACID ANALYSIS OF THE NP
PROTEIN OF SENDAI VIRUS (HVJ)TOSHIHIKO SEMBA¹ and YASUHIRO HOSAKA

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(Received December 21, 1978)

Sendai virus (HVJ) contains three species of inner proteins, designated as M, NP and P. The NP protein constitutes the main component of nucleocapsids (Choppin and Compans, 1975) and forms the transcriptase complex with protein P (Stone et al., 1972), while the M protein is postulated to play a role in association of nucleocapsids with virus envelopes (Choppin and Compans, 1975; Shimizu and Ishida, 1975; Yoshida et al., 1976). In a previous paper (Semba et al., 1977), we reported the isolation and amino acid composition of the M protein. We do not know of any study on the amino acid compositions of the NP and P proteins of the nucleocapsid complex of parainfluenza viruses, including Sendai virus. There are many reports on isolation of paramyxovirus nucleocapsids (Compans and Choppin, 1967; Hosaka, 1968; Kingsbury and Darlington, 1968; Blair and Robinson, 1970; Stone et al., 1972; Waters et al., 1972; Hall and Martin, 1973; Scheid and Choppin, 1973; McSharry et al., 1975),

but polypeptide analysis by SDS-polyacrylamide gel electrophoresis showed that the isolated nucleocapsids contained the other polypeptides, P or M, or both besides the main polypeptide, NP (Stone et al., 1972; Scheid and Choppin, 1973; McSharry et al., 1975; Shimizu and Ishida, 1975).

For amino acid analysis, we tried to isolate the NP protein fraction uncontaminated by other proteins, at the expense of virus-associated activities. The present report describes the isolation of NP protein of Sendai virus by SDS-hydroxylapatite chromatography from a fraction separated from the M protein fraction on Bio-Gel P-200. Chromatography on SDS-hydroxylapatite was carried out as described by Moss and Rosenblum (1972).

Sendai virus, Z strain, was used throughout the present investigation. Preparation of the virus and initial fractionation on a Bio-Gel P-200 column of virus components dissociated with SDS were carried out as described previously (Semba et al., 1977). The second peak (P2) obtained by Bio-Gel P-200 column chromatography was found by SDS-polyacrylamide gel electrophoresis to contain four polypeptides, P, HN, NP and F. The P2

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fraction was dialyzed against 0.01 M sodium phosphate, pH 6.4, containing 0.1% SDS and 0.02 M 2-mercaptoethanol and then adsorbed on a hydroxylapatite (Seikagaku Kogyo Co., Japan, Lot 00275) column equilibrated with the buffer used for dialysis. The column was washed with 1–2 bed volumes of the same buffer, and then materials were eluted with a linear gradient of 0.2–0.4 M sodium phosphate, pH 6.4, containing 0.1% SDS and 0.02 M 2-mercaptoethanol. As shown in Fig. 1, two peaks of material were obtained. SDS-polyacrylamide gel electrophoresis of fractions H1 and H3 showed that the former mainly contained proteins P and HN, while the latter

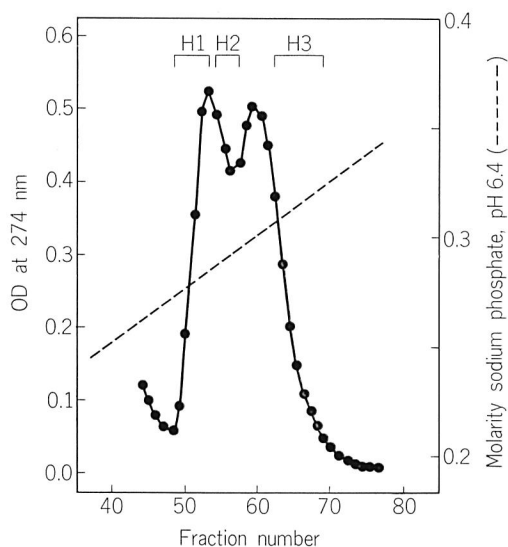


FIGURE 1. SDS-hydroxylapatite column chromatography of fraction P2. Approximately 30 ml of fraction P2 obtained by chromatography of SDS-dissociated proteins from Sendai virus on Bio-Gel P-200 was adsorbed on a hydroxylapatite column (0.9 × 20 cm) equilibrated with 0.01 M sodium phosphate, pH 6.4, containing 0.1% SDS and 0.02 M 2-mercaptoethanol. The material loaded onto the column was eluted at a flow rate of 2.3 ml/hr with a linear gradient of 0.2 M to 0.4 M sodium phosphate (obtained with 120 ml of each buffer). Fractions of 1.4 ml were collected. Fractions H1, H2 and H3, indicated by bars, were analyzed by gel electrophoresis (Fig. 2).

contained only one protein, NP. Fraction H2, between H1 and H3, contained the F protein, besides P and HN (Fig. 2).

The effluent containing fraction H3 was first dialyzed against 0.06 M sodium acetate, pH 5.2, containing 6 M urea and Dowex 1 (acetate form) to trap SDS, followed by dialysis against several changes of distilled water at room temperature. The NP protein usually precipitated nearly completely. The precipitate was collected by centrifugation and dried in vacuo over anhydrous calcium sulfate.

The amino acid composition of the NP

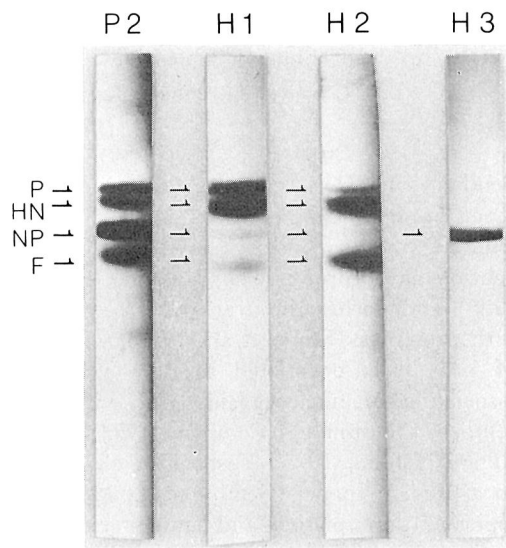


FIGURE 2. SDS-gel electrophoretic analysis of fractions P2, H1, H2 and H3. Electrophoresis was carried out in 7.5% polyacrylamide gels at 5 ma per gel for 6 hr and then the gels were stained by Coomassie brilliant blue. P, HN, NP and F denote molecular species of virus protein (Choppin and Compans, 1975).

protein obtained as described above is shown on the left in Table 1. About 24% (on a weight basis) of the NP protein consisted of acidic residues, and 17% and 32%, of basic and hydrophobic residues, respectively. At present, no information is available about the

TABLE 1. Comparison of amino acid compositions of RNA virus nucleocapsids^a

Amino acid ^b	RNA viruses						TMV ⁴ OM	Semliki Forest virus ⁵
	Sendai ¹ virus	Influenza viruses ²		Newcastle disease viruses ³				
		A(HON)1/ Bel	B/Lee	Ulster	Texas	Beaudette C		
Lysine	6.34	6.55	10.37	1.15	1.44	1.99	1.47	15.04
Histidine	2.27	1.60	1.13	7.64	7.22	6.37	0	3.26
Arginine	8.83	11.63	7.32	7.29	9.10	8.41	9.82	7.09
Aspartate	11.87	8.89	12.69	11.67	9.84	12.58	12.50	8.43
Glutamate	12.23	17.61	18.02	13.35	12.13	15.10	11.81	11.99
Threonine	6.42	5.08	5.57	8.47	7.99	6.39	8.67	5.79
Serine	5.01	5.31	5.38	7.97	5.49	10.45	7.97	4.00
Proline	3.03	3.40	3.48	5.96	5.92	8.18	4.44	7.30
Glycine	5.37	4.20	4.67	ND ^c	ND	ND	1.96	4.77
Alanine	6.34	5.10	5.42	6.63	6.62	8.66	5.69	5.70
Cysteine	(0.0) ^d	0.75	0.0	1.50	1.32	0.80	0.59	1.45
Valine	6.22	4.80	4.73	4.63	5.54	2.12	8.50	6.00
Methionine	2.12	1.65	2.29	6.60	9.17	5.20	0	3.06
Isoleucine	5.18	6.40	7.80	3.83	4.49	2.23	5.18	4.06
Leucine	8.06	8.84	9.98	9.53	7.44	7.46	7.76	3.91
Tyrosine	4.73	2.49	2.69	ND	ND	ND	3.73	3.43
Phenylalanine	4.71	5.68	4.73	3.77	6.31	4.85	6.73	3.19
Tryptophan	0.99	—	—	ND	ND	ND	3.19	1.51

^a Values are expressed on a weight-% basis. ^b Amino acids are grouped as basic, acidic, neutral and hydrophobic from the top. ^c Not determined. ^d See text.

References 1: the present work, 2: Laver and Baker (1972), 3: Moore and Burke (1974), 4: Nozu et al. (1970), 5: Kennedy and Burke (1972).

Sendai virus NP protein was hydrolyzed with 4 N methanesulfonic acid containing 0.2% tryptamine (Simpson et al., 1976). Values for Sendai NP protein are averages of duplicate measurements.

contents of asparagine and glutamine. No cysteine was detectable, suggesting that there are very few intramolecular S-S bonds or SH groups in the NP protein. However, further study on the presence or absence of S-S bonds is necessary.

Table 1 also shows the amino acid compositions of nucleocapsid proteins of other RNA viruses. It is interesting that in all the helical nucleocapsids shown, the content of acidic residues, 24–30%, is higher than that of basic ones (~10%), and there is a rather high content of hydrophobic residues, 30–35%. These

values are different from those for a cubic nucleocapsid of Semliki Forest virus, which has a lower content of acidic residues (20%) than of basic ones (25%), and has 25% of hydrophobic residues. The similarity in the amino acid compositions of the NP protein and other nucleocapsids proteins may reflect common features in helical nucleocapsid construction.

Dansylation of NP protein was carried out by the method described previously (Semba et al., 1977). On chromatography in two different solvent systems, only phenylalanine

was identified as the N-terminus.

ACKNOWLEDGMENTS

The authors wish to thank Prof. K. Fukai (this Institute) for his support and encouragement, and

Prof. Y. Okada and Dr. T. Uchida (this Institute) and Dr. Y. Hosokawa (The Radiation Institute of Osaka Pref.) for their advices. The excellent technical assistance of Dr. S. Kimura and Miss Y. Yagi (Institute for Protein Research) is gratefully acknowledged.

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