

Proteins and Glycoproteins of Paramyxoviruses: a Comparison of Simian Virus 5, Newcastle Disease Virus, and Sendai Virus

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The polypeptides of three paramyxoviruses (simian virus 5, Newcastle disease virus, and Sendai virus) were separated by polyacrylamide gel electrophoresis. Glycoproteins were identified by the use of radioactive glucosamine as a carbohydrate precursor. The protein patterns reveal similarities among the three viruses. Each virus contains at least five or six proteins, two of which are glycoproteins. Four of the proteins found in each virus share common features with corresponding proteins in the other two viruses, including similar molecular weights. These four proteins are the nucleocapsid protein (molecular weight 56,000 to 61,000), a larger glycoprotein (molecular weight 65,000 to 74,000), a smaller glycoprotein (molecular weight 53,000 to 56,000), and a major protein which is the smallest protein in each virion (molecular weight 38,000 to 41,000).

The paramyxoviruses simian virus 5 (SV5), Newcastle disease virus (NDV), and Sendai virus are morphologically similar, and their nucleocapsids, which have each been examined in detail, are remarkably similar in structure and chemical composition (10, 11, 17, 20, 21, 23). Previous reports from this laboratory indicated that there are six polypeptides in the SV5 virion, two of which are glycoproteins (7, 22). One report has described as many as eight polypeptides in the NDV virion (16), whereas others have identified with certainty only three NDV polypeptides (4, 18). This report describes a comparative study of the polypeptides of SV5, NDV, and Sendai virus under identical conditions. The glycoproteins of NDV and Sendai virus have been identified, and the use of a carbohydrate label has permitted the identification of a polypeptide in the NDV virion which was not otherwise detected. The results of these studies, although indicating some differences among the three viruses, also reveal a number of similarities in their protein composition, and thus underscore their morphological and biological relatedness.

MATERIALS AND METHODS

Cells. Monolayer cultures of a variant of the MDBK line of bovine kidney cells were grown on plastic surfaces in reinforced Eagle's medium (REM; reference 1) with 10% fetal calf serum as described previously (9).

Viruses. The W3 strain of SV5 was grown in MDBK

cells as previously described (8, 19). Seed stocks of the Hickman strain of NDV and of Sendai virus were grown in the allantoic sac of 10- to 11-day-old embryonated chicken eggs. Eggs were inoculated with $\sim 10^6$ egg infectious doses (EID₅₀), and allantoic fluid was harvested at 24 hr.

Growth and purification of virus. For studies of virion proteins, all three viruses were grown in MDBK cells in serum-free REM, and released virus was harvested 24 to 42 hr after inoculation with 2 to 20 infective virions per cell. For isotopic labeling of viral proteins, 5 μ Ci of 3 H-leucine or 3 H-glucosamine per ml, or 1 μ Ci of 14 C-amino acid mixture per ml, or both, was added. Cell debris was sedimented at 3,000 \times g for 20 min. The virus was precipitated with an equal volume of saturated ammonium sulfate and banded twice in linear 15 to 40% (w/w) potassium tartrate gradients at 23,000 rev/min for 2.5 hr in a Spinco SW25.1 rotor, dialyzed overnight against REM or deionized distilled water, and either used immediately or stored at -60 C. Nucleocapsid was isolated and purified from detergent-disrupted virus as described previously (23).

Chemicals and isotopes. Components for polyacrylamide gels were obtained from Canal Industrial Corp., Rockville, Md.; 3 H-leucine, 3 H-glucosamine, and 14 C-amino acid mixture from New England Nuclear Corp., Boston, Mass. or from Schwartz BioResearch, Inc., Orangeburg, N.Y.; bovine serum albumin (BSA) from Armour Pharmaceutical Co., Chicago, Ill.; trypsin, two times crystallized, from Worthington Biochemical Corp., Freehold, N.J.; and NCS Solubilizer from Amersham/Searle Corp., Des Plaines, Ill.

Polyacrylamide gel electrophoresis. Procedures employed in the preparation of gels and in electrophoresis have been described (7, 23); 7.5% acrylamide gels, approximately 10 cm long with a 0.5-cm stacking gel of 2.5% acrylamide, were used. Molecular weight estimations were based on migration of the polypeptides relative to BSA and trypsin. For coelectrophoresis experiments, samples were mixed before treatment with sodium dodecyl sulfate and mercaptoethanol.

RESULTS

SV5. The polypeptides of the SV5 virion have been described previously (7, 22) and are shown in Fig. 1 for comparative purposes. Five polypeptides are clearly resolved. A sixth polypeptide, viral protein (VP) 1, with an estimated molecular weight of $\sim 76,000$ (not seen in Fig. 1), is frequently not detected in radioactively labeled gels, although it is seen more consistently in stained gels. It is therefore uncertain that this is a viral protein.

VP3, which accounts for more of the total protein of the virion than any other protein, is now known to be the nucleocapsid protein (23). VP2 and VP4 have been shown to be glycoproteins (22). As shown in Fig. 1, some material is frequently found near the origin on polyacrylamide gel electrophoresis of paramyxoviruses; however, the amount of this material is variable, and the

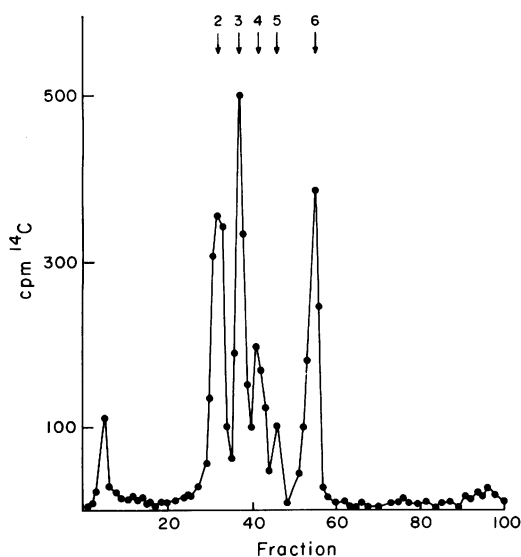


FIG. 1. Polyacrylamide gel electrophoresis of the polypeptides of SV5. Virions labeled with ^{14}C -amino acids were disrupted with sodium dodecyl sulfate and mercaptoethanol and subjected to electrophoresis for 14 hr. VP1 was not detected in this experiment. In this and subsequent figures, the origin is on the left and the anode on the right.

available evidence suggests that it represents aggregation rather than a very large viral protein (7). As shown below, such aggregation is sometimes particularly prominent with NDV (Fig. 3), but again the amount is variable (cf. Fig. 3 and 7).

NDV. Figure 2 illustrates the electrophoretic pattern of NDV virion proteins labeled in the presence of ^3H -leucine. At least six protein species can be identified by using both radioactively labeled and stained gels. Three of these protein species (VP1, 3, and 6) are present in relatively large amounts and have been described by others (4, 16, 18). Our estimates for the molecular weights of these major proteins are $\sim 74,000$, 56,000, and 41,000 (Table 1). VP3, which is the most prominent, is the nucleocapsid protein of the NDV virion (4, 16, 18, 23). A small peak labeled with a question mark in Fig. 2 was frequently (cf. Fig. 7), but not always, found in our experiments. A band in this position was also found by Evans and Kingsbury (16). We consistently found VP4, either as a distinct peak (Fig. 2) or as a shoulder on the leading edge of VP3 (Fig. 3). VP5 was always found as a small peak between VP4 and a major peak, VP6. Evans and Kingsbury (16) found one minor protein between the two major peaks which correspond to our VP3 and VP6.

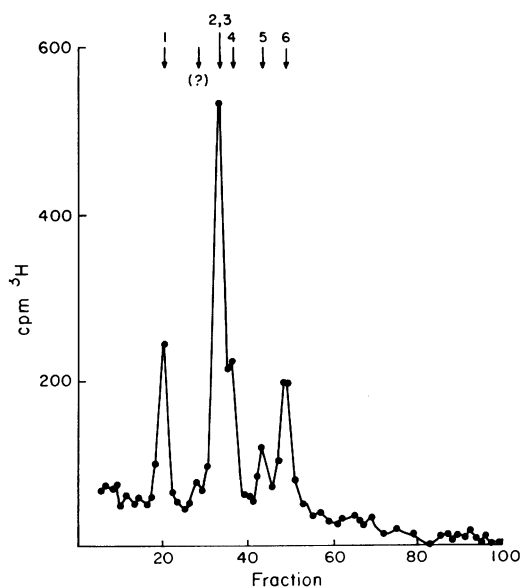


FIG. 2. Electrophoretic pattern of NDV virion proteins labeled with ^3H -leucine. Two proteins, VP2 and VP3, migrate together (see also Fig. 3). The unnumbered arrow indicates a small peak which is not always detected and therefore its status as a viral protein is uncertain.

In previous studies with Sindbis virus in chick embryo fibroblasts (6) and SV5 in MDBK cells (22), glucosamine was found to be a specific label for carbohydrate linked to viral proteins. When NDV was grown in the presence of ^3H -glucosamine and ^{14}C -amino acids, two polypeptides were identified as glycoproteins, i.e., VP1 and VP2 (Fig. 3). The latter migrates almost identically with VP3, the nucleocapsid protein; however, the nucleocapsid protein contains no glucosamine. This was shown by isolating and purifying the nucleocapsid from a sample of the same virus preparation used in the experiment shown in Fig. 3. On gel electrophoresis of this purified nucleocapsid protein, the single polypeptide peak contained 1,600 ^{14}C disintegrations per min, but no ^3H disintegrations per min. (In Fig. 3 the amounts of radioactivity in amino acids and glucosamine are similar in the region of VP2, 3.) Thus, VP2 is not identical to the nucleocapsid protein, but is a glycoprotein with a similar electrophoretic mobility which can be detected only by labeling with a carbohydrate precursor. In summary, six polypeptides including two glycoproteins have been definitely identified in NDV virions; the presence of a seventh protein is questionable.

Sendai virus. The radioactive pattern obtained on electrophoresis of the polypeptides of Sendai virions labeled with ^{14}C -amino acids is shown in Fig. 4. At least six, and possibly seven, polypeptide species can be identified. Three proteins,

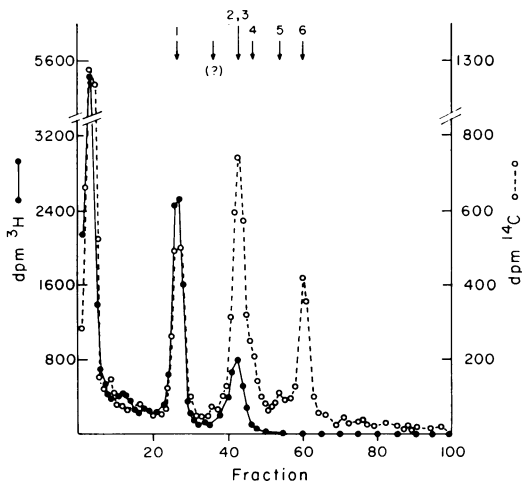


FIG. 3. Identification of the glycoproteins of NDV. Virions grown in the presence of ^3H -glucosamine and ^{14}C -amino acids were disrupted and subjected to electrophoresis. The presence of ^3H -label indicates that VP1 and VP2 are glycoproteins. VP2 migrates nearly identically with the nucleocapsid protein (VP3), which contains no carbohydrate. VP4 is present as a shoulder on the leading edge of VP3.

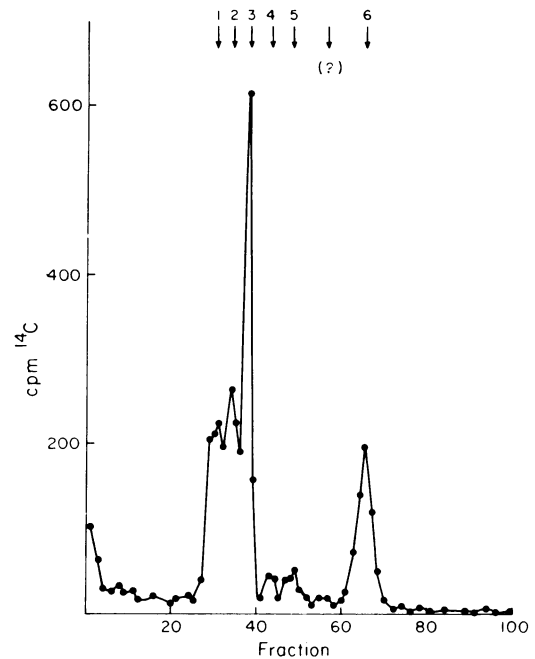


FIG. 4. Electrophoresis of Sendai virus proteins labeled with ^{14}C -amino acids. VP1, 2, 3, and 6 are prominent protein species. VP4 and 5, although quite small in amount, are always seen. The unnumbered arrow indicates a tiny peak which may not represent an essential viral protein.

present in large amounts (VP1, 2, 3) migrate quite closely together, with molecular weights of $\sim 69,000$, $65,000$, and $60,000$ (Table 1). As with SV5 and NDV, the most prominent protein (VP3) is the nucleocapsid protein (23). A fourth major protein (VP6) is much smaller, with a molecular weight of $\sim 38,000$. Two proteins of intermediate size (VP4 and VP5) are present consistently, but in very small amounts. There is a third very minor component with a molecular weight of $\sim 46,000$ which conceivably represents an enzymatic cleavage product of the nucleocapsid subunit (23) and therefore may not be an essential viral protein.

The glycoproteins of Sendai virions (VP2 and VP5) are identified by labeling with ^3H -glucosamine (Fig. 5). Preliminary data suggest that treatment of Sendai virions with Pronase removes all of the glucosamine label, and the particles still contain most or all of the protein designated as VP1. These findings have been interpreted to mean that the glucosamine counts under VP1 in Fig. 5 probably represent overlap from VP2. An alternative explanation, which appears less likely, is that there may be two viral proteins under the peak designated VP1, one a glycoprotein and one a nonglycoprotein. Further experiments will be required to exclude this latter possibility.

TABLE 1. Molecular weights of the polypeptides of SV5, Newcastle disease virus, and Sendai virus^a

SV5		NDV		Sendai virus	
Protein no.	Molecular wt	Protein no.	Molecular wt	Protein no.	Molecular wt
1 (?) ^b	76,000	1	74,000	1	69,000
2	67,000	(?)	62,000	2	65,000
3	61,000	2	56,000	3	60,000
4	56,000	3	56,000	4	58,000
5	50,000	4	53,000	5	53,000
6	41,000 ^c	5	46,000	(?)	46,000
		6	41,000	6	38,000
Total	351,000		388,000		389,000

^a Molecular weight estimates are based on migration in polyacrylamide gels relative to bovine serum albumin and trypsin. Similar values were obtained for NDV and Sendai virus protein when SV5 proteins were used as markers.

^b Question mark (?) indicates a protein whose status as a viral protein is uncertain.

^c Molecular weight of SV5 VP6 was previously estimated as ~43,000 (7); the present revised value of 41,000 is based on the average of 14 different experiments.

Coelectrophoresis studies. A more critical comparison of the protein patterns of these three paramyxoviruses is obtained by mixing SV5 with either of the other two viruses and running them together in the same gel. Figures 6 and 7 represent such experiments. The arrows at the top of the figures represent the peak fractions of each of the various proteins in the two virions. Figure 6 shows the similarity between SV5 and Sendai virus, with respect to both the general pattern and the molecular weight of the proteins as revealed by migration in the gel. There is less similarity between SV5 and NDV (Fig. 7). Coelectrophoresis of NDV and Sendai virion proteins showed a degree of correspondence of the two patterns which was similar to that shown in Fig. 7.

Table 1 summarizes the estimates of the molecular weights of the proteins in the three viruses. These values are based on relative migration in gels with BSA and trypsin as markers. However, the coelectrophoresis experiments allowed the use of SV5 proteins as markers. Values obtained for NDV and Sendai proteins with SV5 proteins as markers varied no more than 1,000 daltons from those obtained with BSA and trypsin markers, and the use of SV5 proteins allowed a more precise estimate of the molecular weight of Sendai proteins VP1 and VP2, which are partially obscured by BSA in stained gels.

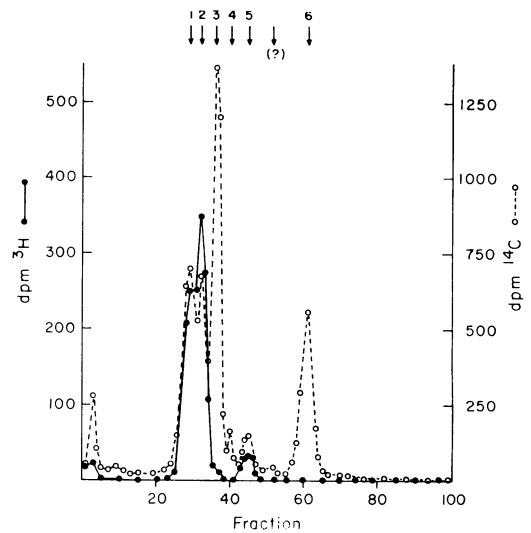


FIG. 5. Identification of Sendai virus glycoproteins. Sendai virion proteins were labeled with ³H-glucosamine and ¹⁴C-amino acids, and the virions were disrupted and subjected to electrophoresis. VP2 and VP5 are seen to be glycoproteins. VP1 probably does not contain carbohydrate. (see text).

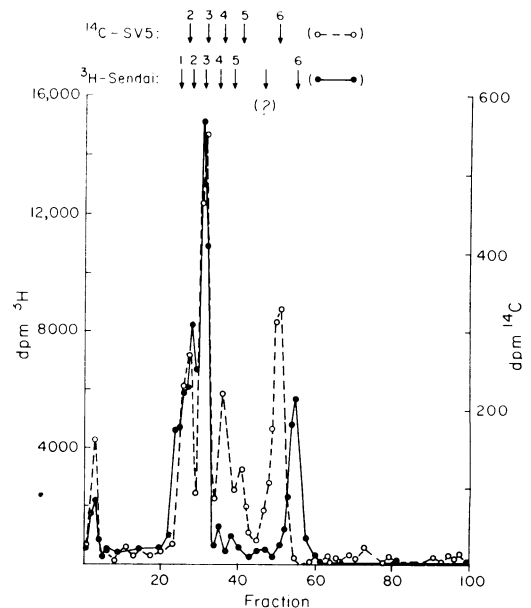


FIG. 6. Coelectrophoresis of SV5 proteins labeled with ¹⁴C-amino acids and Sendai virus proteins labeled with ³H-leucine.

DISCUSSION

The similarities in morphology and chemical composition of paramyxoviruses suggested that similarities would also be found in their poly-

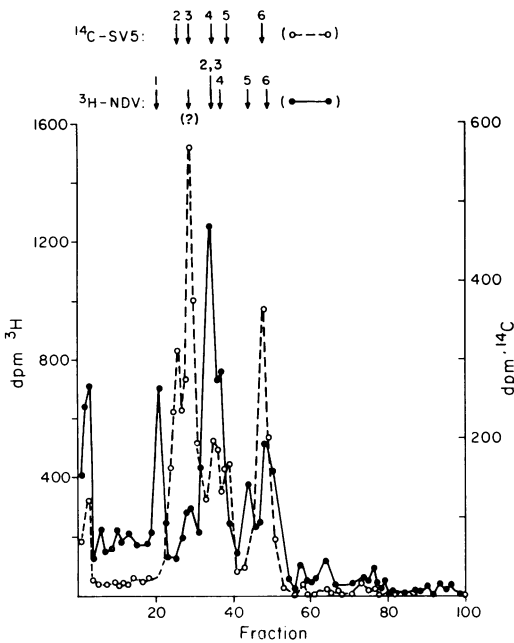


Fig. 7. Coelectrophoresis of SV5 proteins labeled with ¹⁴C-amino acids and NDV proteins labeled with ³H-leucine.

TABLE 2. Similarities in the molecular weights of four types of proteins which are found in paramyxoviruses

Protein	SV5	Sendai virus	NDV
Larger glycoprotein...	67,000	65,000	74,000
Nucleocapsid protein...	61,000	60,000	56,000
Smaller glycoprotein...	56,000	53,000	56,000
Smallest protein.....	41,000	38,000	41,000

peptide components. The present study indicates that such similarities exist among SV5, NDV, and Sendai virus, as indicated in Table 2. Each virus contains three or four polypeptide species which are present in relatively large amounts, and in each case the most prominent protein species of the virion is the nucleocapsid protein. Further, the nucleocapsid proteins of the three viruses have similar molecular weights (~ 56,000 to 61,000). The smallest polypeptide of each virion is also a major protein which has a molecular weight near 40,000. Each of the three viruses possesses two glycoproteins, and in each case the larger glycoprotein represents a greater proportion of the total viral protein than does the smaller glycoprotein. Finally, all three viruses possess two or three protein species which are present in relatively minor quantities.

The total estimated molecular weights of all the polypeptides in the three viruses, including the questionable minor ones, are: SV5, 351,000; NDV, 388,000; and Sendai virus, 389,000 (Table 1). The ribonucleic acid genome of each virus has been estimated to be 6×10^6 to 7×10^6 daltons (2, 12, 15) which could code for 600,000 to 700,000 daltons of protein. Thus, with each virus the calculated totals for the virion polypeptides would leave sufficient genetic information to code for several nonstructural proteins.

The assignment of biological function to the virion proteins is far from complete. The only one identified with certainty for each virus is the nucleocapsid protein (4, 16, 18, 23). By using partially purified fractions of disrupted NDV particles, Haslam and co-workers (18) tentatively associated the hemagglutinating activity with their viral protein 1, which is probably identical with our NDV VP1, the larger glycoprotein. This protein was also found by Evans and Kingsbury (16) to be the major component of an NDV fraction which adsorbed to and eluted from erythrocytes. Haslam and co-workers (18) reported that NDV neuraminidase activity was associated with their partially purified protein 3 (our VP6). However, since they did not resolve the three minor components of the NDV virion, it is possible that these "minor" polypeptides, either alone or in concert with other polypeptides, may be associated with hemagglutinating and neuraminidase activities. Chen, Compans, and Choppin (*manuscript in preparation*) have found that the SV5 glycoproteins, VP2 and VP4, are absent in virions from which the surface projections or spikes have been removed by treatment with a proteolytic enzyme, and the spikeless particles have lost their hemagglutinating and neuraminidase activities. These findings suggest that these two activities are associated with the two spike glycoproteins, and the fact that VP4 is present in relatively small amount emphasizes the importance of such "minor" proteins. These studies have also suggested that the smallest protein in the SV5 virion, VP6, may be the major structural protein of the viral membrane. Similar studies with NDV and Sendai virus are in progress.

The present results indicate that there are similarities in the pattern of the proteins of three paramyxoviruses. They also emphasize the presence of minor proteins, particularly in NDV, where the three major protein peaks described previously (4, 16, 18) were found, but other proteins were also clearly resolved, including a glycoprotein that could be identified only by the use of a carbohydrate precursor. The presence of glycoproteins in each of the three paramyxoviruses is in accord with the results with several other en-

veloped viruses (3, 5, 6, 14, 24, 26–28), and, in those cases in which the nature of the surface projections has been determined, these projections have been found to be glycoproteins (13, 25; R. W. Compans, *submitted for publication*).

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