

## Fish Rhabdoviruses: Comparative Study of Protein Structure

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Proteins from four fish rhabdoviruses have been studied by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The viruses were: trout viral hemorrhagic septicemia (VHS), infectious hematopoietic necrosis virus (IHN), spring viremia virus of carp (SVC), and the pike fry rhabdovirus (PFR). For the two salmonid viruses (VHS-IHN), gel electrophoresis indicated the proteins, with molecular weights estimated to be 190,000, 80,000, 38,000, 25,000, and 19,000, respectively. The electrophoretic profile of the two other viruses (SVC-PFR) revealed four major proteins with molecular weights of 190,000, 80,000, 42,000, and 21,000, respectively. In this case a minor component with 50,000 daltons was found. For each virus only one protein was found to be glycosylated, i.e., the one with a molecular weight of 80,000. A major protein (molecular weight between 38,000 and 42,000) was found to be associated with the nucleocapsid. All these results revealed marked similarities in protein structure between the four fish rhabdoviruses and the previously well-characterized members of rhabdovirus group. However, one can distinguish two groups of viruses: the first one is composed of salmonid viruses (VHS and IHN) with a protein structure comparable to that of rabies virus and potato yellow dwarf virus; the second one is composed of carp and pike viruses, having a protein structure very similar to that of vesicular stomatitis virus.

Four fish viruses, which may be classified among rhabdoviruses, are presently known to cause specific diseases in fish. Two of the viruses are pathogenic for salmonids, namely the viral trout hemorrhagic septicemia virus (VHS) or Egtved virus (19) and the infectious hematopoietic necrosis virus (IHN) (1). Two variants are serologically and morphologically related to IHN: the Chinook salmon virus and the Oregon sockeye virus (2, 11). The latter two viruses have not been examined because of their known similarity to IHN. The other two viruses are spring viremia virus of carp (SVC) (3) and the pike fry rhabdovirus (PFR) (6).

The protein composition of SVC (10) and PFR has been previously reported (7, 13). In this communication we describe the protein composition of the two salmonid viruses, VHS and IHN, the partial localization of the different proteins in the virions and the presence of glycoproteins in the four types of viruses.

### MATERIALS AND METHODS

The viruses were grown in monolayer cultures of fathead minnow cells (4) as previously described (5, 7,

10). Virus stocks were produced using diluted inoculum (input multiplicity: 0.01 PFU/cell) to avoid both production of defective particles and induction of interferon. Infectious cell culture fluids were clarified by 10 min of centrifugation at 4,000 × g and concentrated by polyethylene glycol precipitation. Purification of the viruses was achieved by isopycnic sucrose gradients as formerly shown (10). In most experiments stocks of labeled virus had been produced using a <sup>14</sup>C-labeled amino acid mixture (30 mCi/matom of carbon) or D-[<sup>14</sup>C]glucosamine (45 mCi/nM).

Before electrophoresis, purified virus or viral components were disrupted by treatment with 1% sodium dodecyl sulfate and 0.5 M urea at 100 C for 2 min. The samples were then dialyzed overnight against 0.01 sodium phosphate buffer (pH 7.1), containing 0.1% sodium dodecyl sulfate, 0.5 M urea, and 0.1% 2-mercaptoethanol. The samples were then layered onto a 7% acrylamide gel (linked with ethylene diacrylate) prepared and run as described previously (9, 10). Gel slices were made using a Gilson slicer and counted after the addition of 10 ml of Brays scintillation fluid in a Packard Tri-Carb spectrometer.

Stepwise dissociation of the virion was performed by using Triton X100 at concentrations varying between 0.1 and 2% (vol/vol), and nucleocapsid preparation by using 0.1% (vol/vol) of sodium desoxycholate. In every case, virions purified as described previously were suspended in 10 mM Tris buffer (pH 7.6) containing 150 mM NaCl and treated for 30 min at

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room temperature. The particulate components were then pelleted at 50,000 rpm for 45 min in a Spinco SW65 rotor, resuspended in 1:10 of the initial volume in 10 mM sodium phosphate buffer (pH 7.2), and analyzed by polyacrylamide gel electrophoresis as previously described.

Molecular weight determination was made according to Shapiro et al. (14) using as protein standards: bovine serum albumin (66,000 daltons); human gammaglobulin (50,000 and 25,000 daltons); pepsin (35,000 daltons); and trypsin (24,000 daltons).

## RESULTS AND DISCUSSION

Polyacrylamide gel analysis of viral polypeptides shows the similarity of the electrophoretogram obtained, between SVC and PFR and between VHS and IHN (Fig. 1).

One can observe in the case of SVC and PFR (Fig. 1a and b) the presence of four major proteins having a molecular weight of 190,000, 80,000, 42,000, and 21,000, respectively (Table I). In these two cases a minor component having a molecular weight of 50,000 can also be found.

With VHS and IHN (Fig. 1c and d), however, five proteins having a molecular weight of 190,000, 80,000, 38,000, 25,000 and 19,000 can be observed (Table 1) without any detectable minor component.

The data obtained with SVC and PFR was

identical, as was the data between VHS and IHN. Only the results obtained with SVC and VHS are shown in Fig. 2 and 3.

When the viruses were grown in the presence of [ $^{14}\text{C}$ ]glucosamine (Fig. 2a and b), the radioactivity peak was found to migrate with the protein having molecular weight of 80,000.

Analysis of the viral nucleocapsids obtained after deoxycholate treatment (Fig. 2c and d) revealed only one polypeptide, the major one, with a molecular weight varying between 38,000 and 42,000 according to the type of virus. Electron microscopy showed nucleocapsids free of other virion components (J. Cohen and G. Lenoir, *Ann. Resch. Vet.*, in press).

According to Wagner et al. (17) the proteins of rhabdoviruses may be classified as: L for the large protein, G for the glycoprotein, N for the protein associated with the nucleic acid, and M for the membrane or matrix protein(s) with lower molecular weight.

All four viruses had the L protein. However, this protein is never found to be associated with the nucleocapsid prepared after treatment of purified virus with sodium deoxycholate, contrary to what has been described for vesicular stomatitis virus (18).

The four viruses also have a G glycoprotein as

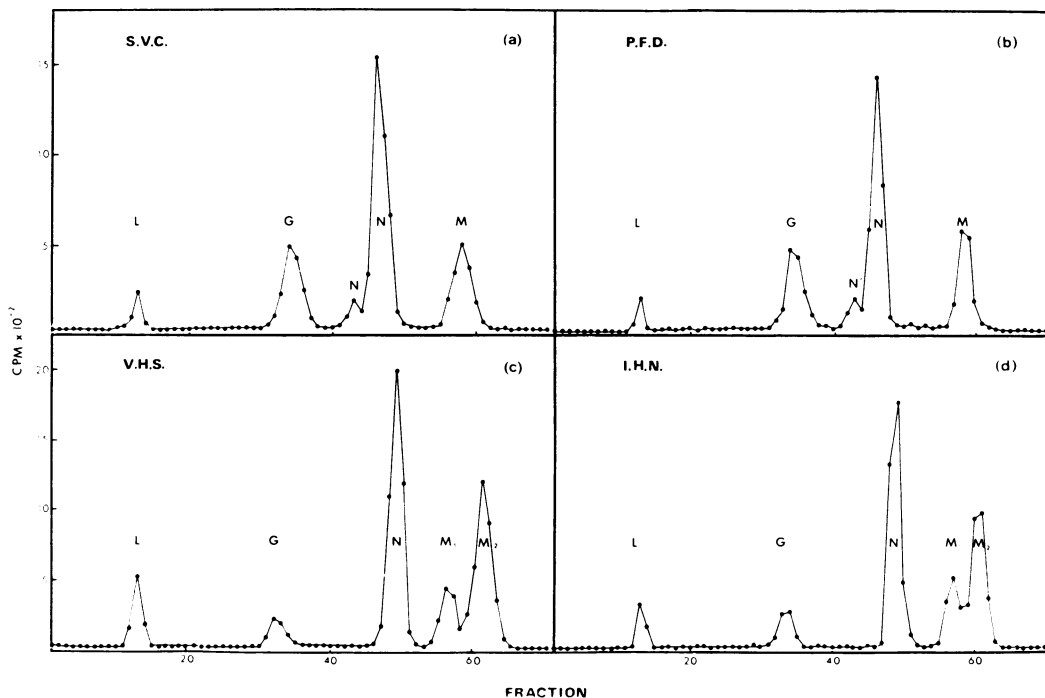


FIG. 1. Sodium dodecyl sulfate-polyacrylamide gel analysis of purified virion polypeptides labeled with  $^{14}\text{C}$ -labeled amino acids of: (a) spring viremia virus of carp, (b) pike fry rhabdovirus, (c) viral hemorrhagic septicemia virus, and (d) infectious hematopoietic necrosis virus. Migration was from left to right.

TABLE 1. *Molecular weights of the polypeptides of fish rhabdoviruses*

Proteins <sup>a</sup>	L	G	N	M1	M2
VHS and IHN	190,000	80,000	38,000	25,000	19,000
SVC and PFR	190,000	80,000	42,000	21,000	

<sup>a</sup> Nomenclature according to Wagner et al. (17).

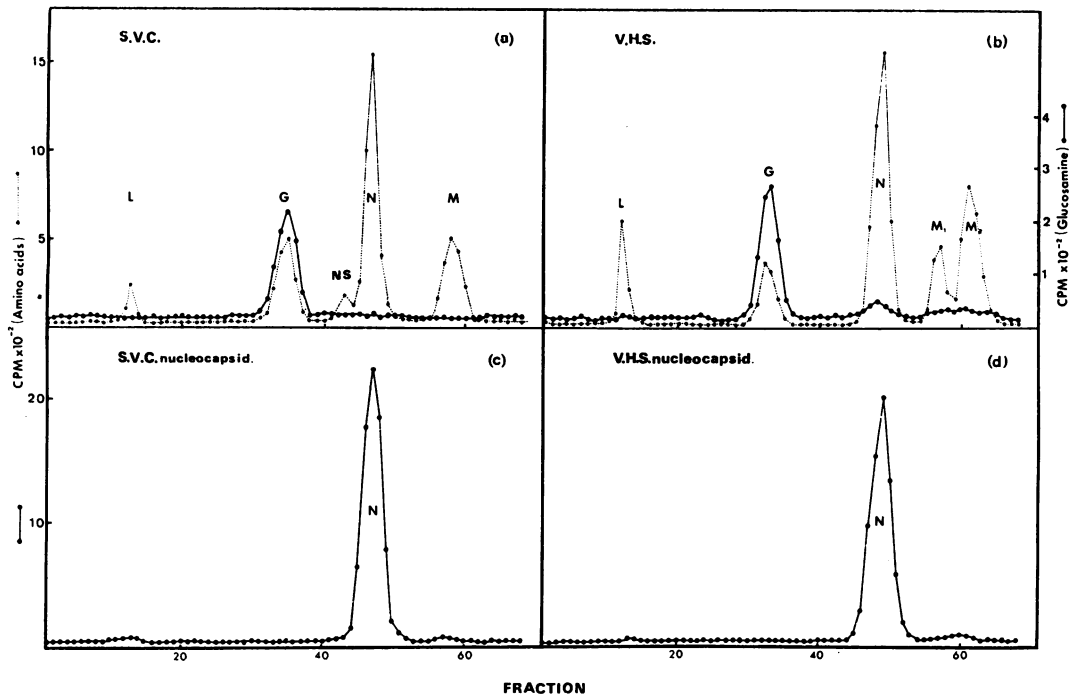


FIG. 2. *Electrophoresis in polyacrylamide gel of: (a) [<sup>14</sup>C]glucosamine-labeled SVC, ●—● (run in parallel with <sup>14</sup>C-labeled amino acid-labeled SVC, ●—●); (b) SVC nucleocapsid; (c) [<sup>14</sup>C]glucosamine-labeled VHS, ●—● (run in parallel with <sup>14</sup>C-labeled amino acid-labeled VHS, ●—●); and (d) VHS nucleocapsid. Migration was from the left to the right.*

can be shown by labeling with [<sup>14</sup>C]glucosamine. As is the case of the other members of rhabdovirus group, this glycoprotein, which can be removed by proteolytic treatment (not shown), is the component of the spikes present on the surface of the virion (Cohen and Lenoir, *Ann. Resch. Vet.*, in press). This glycoprotein is less detectable on the electrophoretogram of the two salmonid viruses (IHN-VHS). This is consistent with the difficulty to characterize the spikes of the two viruses by electron microscopy (Cohen and Lenoir, *Ann. Resch. Vet.*, in press) as well as their low immunogenic potential (P. de Kinkelin and M. Dorson, personal communication).

Through deoxycholate treatment we obtained a nucleocapsid composed of the viral nucleic

acid and the N protein. Through a stepwise degradation of the virions with Triton X100 we were able to remove, first, the glycoprotein and, second, the large protein L and the M protein for SVC and PFR (Fig. 3a). Figure 3b reveals that in the case of VHS and IHN, after removal of the G protein, L and M<sub>2</sub> proteins are removed whereas M<sub>1</sub> seems to be mostly associated with the nucleocapsid similar to potato yellow dwarf virus (8).

The SVC and PFR viruses possess a minor polypeptide N' migrating more slowly than the N protein. This protein is certainly the same as the one named nonstructural for the vesicular stomatitis virus but the migration seems to vary according to the conditions of gel electrophoresis as described by Stampfer and Baltimore (16)

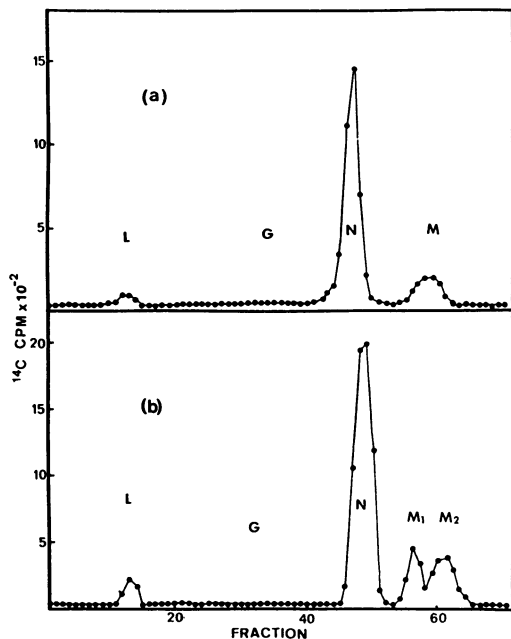


FIG. 3. Electrophoretic profiles of the proteins of the particulate components obtained after treatment of (a) SVC and (b) VHS by 1% Triton X100. Purified  $^{14}\text{C}$ -labeled amino acid-labeled virions, suspended in 10 mM Tris buffer (pH 7.6) containing 0.15 M NaCl, were treated by 1% Triton X100 for 30 min at room temperature. The particulate components were then pelleted at 50,000 rpm for 45 min in a Spinco SW65 rotor, resuspended in 1:10 of the initial volume in phosphate buffer, and analyzed by polyacrylamide gel electrophoresis as described in the text. Migration was from left to right.

and Obijeski et al. (12).

The comparative study of protein structure of these four fish rhabdoviruses permit us to establish two groups of viruses: the salmonid viruses (VHS and IHN) on one hand, having a protein structure very similar to that of rabies (15) and potato yellow dwarf virus and, on the other hand, the pike and carp viruses, being very similar to vesicular stomatitis virus in terms of general protein structure.

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