STRUCTURAL PROTEINS OF SPRING VIREMIA VIRUS OF CARP

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SUMMARY :

Spring viremia virus (S.V.V.) of carp produced on fathead minnow cells (FHM) has been concentrated by polyethylene glycol and purified by a two step gradient centrifugation. The virus particle has a density of 1.16 in sucrose. Purified S.V.V. was disrupted by sodium dodecylsulfate (SDS),urea, 2-mercaptoethanol and heat, and the proteins were analysed in polyacrylamide gels in presence of SDS. Four different polypeptide chains A, B, C, D were found with M.W. of approximately 150,000, 70,000, 40,000, 19,000 daltons respectively. The three major proteins B, C, D are in molar ratio of 1:4:4.

INTRODUCTION :

From a morphological view point several fish viruses belong to the rhabdovirus group : Egtved virus (18), infectious hematopoietic necrosis virus (1), the agent of red disease of pike (7) and the etiological agent of the acute form of infectious dropsy (Spring viremia) of carp (8). The purpose of this work is the study of S.V. virus protein structure, number and size of the peptides of the viral particle.

METHODS :

Fathead minnow cells (F.H.M.) (9) were propagated as previously described by de Kinkelin (5).

Virus was supplied by Dr Fijan (Zagreb).

Cells were infected at an input multiplicity of 0.1 pfu/cell in Eagle's medium supplemented with 2% foetal calf serum ; virus was labelled using 1 μCi/ml of a mixture of ^{14}C labelled amino acids (C.E.A. Saclay, France).

After 14 hours at 21°C, the culture fluids were pooled, centrifugated at 2,000 g for 10 min to remove cell debris and then precipitated by adding 7 % polyethylene glycol 6,000 (PEG) and 2.3 % NaCl. After 2 hours at 4°C the precipitate was pelleted at 8,000 g for 30 min and then resuspended in T. Buffer (0.15 M Tris-HCl pH 7.6, 0.15 M NaCl, 1 % EDTA) (6).

In a first step, virus was purified by centrifugation through a 5-20 % sucrose gradient in T. buffer for 90 min at 24,000 rev/min, 4°C, in a Spinco SW 25-I rotor. For further purification of labelled material isopycnic banding of the peak fractions from the previous sucrose gradient was performed ; pooled peak fractions were layered on a linear 15-60 % sucrose gradient in T. buffer and centrifuged in a Spinco SW 25-I rotor for 16 hours at 20,000 rev/min at 4°C.

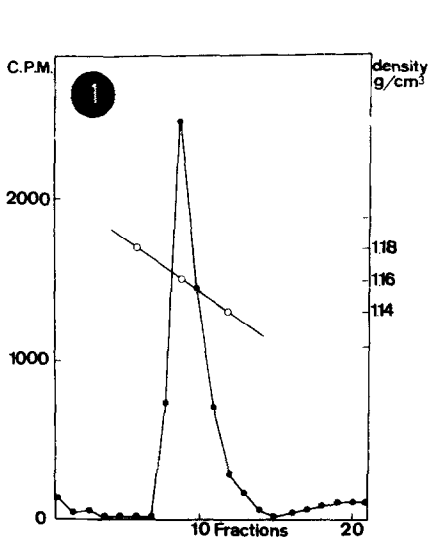
Virus dissociation was performed by heating at 100°C for 1 min in buffer containing 1 % SDS and 0.5 M urea ; the samples were then dialysed overnight against 0.01 sodium phosphate buffer containing 0.1 % SDS, 0.5 M urea and 0.1 % 2-mercaptoethanol.

The samples were layered over 7 cm long, 7.5 %, polyacrylamide gels according to Summers (15) modified by Laporte (12). After migration for 7 hours at 8 mA/gel, the gels were frozen, 1 mm slices were cut and dissolved in NH_4OH. 10 ml Bray's solution (3) was added prior to counting in a Packard scintillation counter. With globulin markers, gels were stained for 1 hour with 0.01 % Coomassie blue solution containing 12.5 % trichloracetic acid.

RESULTS AND DISCUSSION

In sucrose density gradients the purified virus bands at about 1.16 g/cm^3 (Fig. 1) ; these results are in agreement with those obtained with many enveloped viruses.

Four polypeptides chains, designated here A, B, C, D according to decreasing M.W., were repeatedly revealed in purified S.V. virus preparations. The first peak A, was always obtained even if the denaturing conditions were increased by using higher concentrations of either SDS



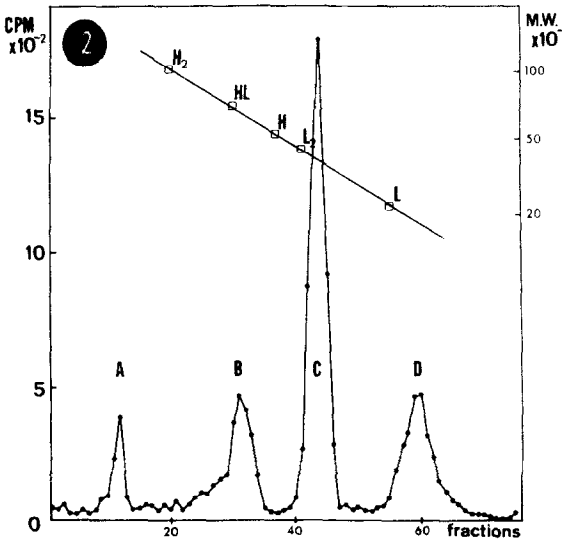


Fig. 1 : Isopycnic banding of labelled S.V.V. Centrifugation was for 16 hours at 20,000 rpm and 4°C in a SW 25-I rotor. Samples of 100 µl were mixed with 10 ml of Bray's solution for counting.

Fig. 2 : S.V.V. proteins labelled with ^{14}C amino acids were prepared as described under methods and analysed by electrophoresis in 7.5% polyacrylamide gels in 0.1 M sodium phosphate buffer pH 7.4, 0.1% SDS. Migration from left to right.

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or urea. Peak A, therefore cannot be considered as an aggregate. The M.W. of the S.V.V. proteins were determined by comparing their electrophoretic mobilities to that of human immunoglobulin G chains, since it is known that in the neutral pH SDS polyacrylamide gels electrophoretic mobility is inversely related to the log of M.W. (14). Under these conditions human immunoglobulin G molecules are partially dissociated so that light and heavy chains can be found as either monomers or dimers, or half molecules (HL). Assuming that the M.W. of heavy and light chains of human immunoglobulins are respectively 52,000 and 22,500 ; the M.W. of the different virus peptides are : 150,000 , 70,000 , 40,000 , 19,000 respectively. Considering the molecular weight of a component, its molar ratio is determined by measuring its proportion in the virion ; such measurements have been attempted from the electrophoretic feature of disrupted virus, uniformely labelled with a ^{14}C amino acids mixture. The area of each peak was taken to be proportional to portein mass. It appears then that the three proteins B, C, D are present in a molar ratio of 1:4:4.

These results show evidence that the S.V.V. structural proteins are very similar to those of V.S.V. (10, 13, 17) since the four proteins A, B, C, D could be related to the L, G, N, M proteins of V.S.V. (16). Protein composition of the rhabdoviruses seems to be very similar whatever the host ; warm-blooded animals, fishes or plants (11). However we never observed on our gels a polypeptide corresponding to NS. Additionnaly, in S.V.V. the molar ratio of the structural proteins is quite different from those given by Cartwright (4) and Bishop (2).

The nucleic acid which can be extracted from this virus is a ribonucleic acid (Lenoir in preparation).

Our results concerning the structural peptides and the viral nucleic acid permit us to confirm that S.V.V. can be classified in the rhabdovirus group.

Studies now in progress are designed to localize the four peptides by stepwise degradation of S.V. virion.

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