STRUCTURAL PROTEINS OF SWINE VESICULAR DISEASE VIRUS

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The physical and chemical properties of foot-and-mouth disease and swine vesicular disease viruses have been compared, and it is concluded that these viruses are structurally different. The purified S.V.D. and F.M.D. viruses were disrupted and analysed in polyacrylamide gels. Two different polypeptide chaines with molecular weight of approximately 38000 and 30000 daltons respectively were found in the virions as well as in the empty capsids of S.V.D. In contrast to FMDV, there was no observable cleavage proteins of the empty capsid yielling detectable shortened polypeptides, which may be associated with the viral RNA .

INTRODUCTION

The recent outbreaks of swine vesicular disease (S.V.D.) in Europe were so typical of foot-and-mouth disease, that the diagnosis was confirmed on the basis of the observed lesions . However after repeated serological tests it appeared that the agent responsible might be different from foot-and-mouth disease virus (F.M.D.V.). Infact, it belongs to the enterovirus subgroup of the picornaviruses (1) . There has not yet been time to complete the study of this new virus. In general its characteristics seem to be rather different from those earlier described for foot-and-mouth disease virus.

The present study was undertaken to define the structural polypeptides of the purified S.V.D. virions, to compare its structural components with those of the foot-and-mouth disease virus and to define the polypeptide composition of purified non infectious empty particles .

MATERIELS AND METHODS

Viruses : The S.V.D.V. was obtained from Dr DHENNIN (2) who isolated this french strain from an outbreak in pigs in the fields around BORDEAUX.

It was grown in pig kidney (IBRS'2) cell line, and high titers were obtained, sometimes exceeding 10^9 plaque forming units per ml . Plaque isolates of S.V.D.V. were labeled by adding a solution containing salts, vitamins and 0,2 Ci/ml of a (14 C) labeled amino-acid mixture to washed cultures 1 hour following infection at a multiplicity of 30 PFU/cell. The viruse was harvested at 16 hours, precipitated with 10 % polyethylene glycol (PEG 6000), resuspended in Tris-HCl buffer (0,01 M tris, 0,001M EDTA, 0,15 M Nacl pH 7,2) and sedimented in a Sw 24 MSE rotor for 16 hours at 20.000 rpm in 15-45 % sucrose gradients in Tris Buffer . The centrifuged gradients were collected and each fraction of 3 ml analysed at 260 nm and titred by complement fixation . Each peak was pooled and dialysed over night against Tris-HCl buffer and purified by CsCl density gradient centrifugation .

The F.M.D.V. mutant O type was grown in the baby hamster kidney cell line BHK-21 clone 13, labeled pratically in the same conditions as described above, precipited with PEG, and purified by three successive density gradient centrifugations .

The light scattering bands of materials were collected and examined by electron microscopy for the presence of virus particles, and for the polypeptide analysis .

Polypeptide analysis in polyacrylamide gel electrophoresis : Virus dissociation was performed by heating at 100°C for 1 mn in buffer containing 1 % S.D.S. and 0,5 M urea ; the samples were then dialysed overnight against 0.01 sodium phosphate buffer containing 0.1 % S.D.S., 0.5 M urea and 0.1 % , 2 mercaptoethanol .

The samples were layered over 7 cm long 10 % polyacrylamide gels and analysis performed according to Summers (3) .

RESULTS AND DISCUSSION

1°) Purification

Concentrated S.V.D.V. was sedimented in sucrose gradients and cosedimented with F.M.D.V. Two well separated peaks of S.V.D.V. antigens were characterized by complement fixation as well as absorbance .

The former which contained about 60 % of the imput material was recovered in the fractions of the gradient at 38 % of sucrose .

The latter which contained only 40 % of the antigenic material was recovered at 25 % of sucrose .

In the cosedimented experiment (fig.1a), F.M.D.V. (140 S) (4-5) sedimented more slowly than S.V.D.V. (150 S) (6-7).

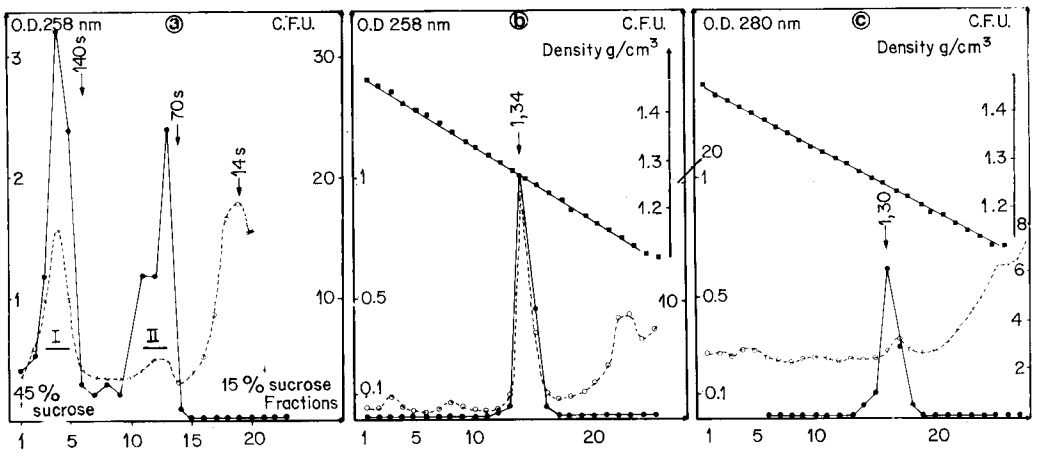

FIGURE 1
a) - Sucrose density gradient sedimentation of PEG precipitated viral particles . The material was centrifuged in a 15 to 45 % sucrose gradient in the MSE rotor Sw 24 for 16 h at 20000 r.p.m. Only the positions of the three F.M.D.V. particles are indicated by the arrows .
b) - Isopycnic banding of S.V.D. virions (population I)
c) - Isopycnic banding of empty capsides (population II). Centrifugations b and c were for 16 h at 55000 r.p.m. in the MSE rotor Sw 65 at 4°C.
O----O optical density
●——● CFU (complement fixation unit.)
■——■ density g/cm3

After dialysis the two populations of antigenic material were banded in isopycnic CsCl gradients. The heavy particles (peak I) recovered in a single narrow band (fig.1b), density 1,34 g/cm3, were infectious . The light particles (peak II) banded at the density of 1,30 g/cm3 were poorly infectious . The light scattering bands were examined with the electron microscope ; the band (d = 1,34 g/cm3) contained complete particles, and some empty particles (10 %) . The light band (d = 1,30 g/cm3)contained only empty partieles . All these particles posessed the same size (30-32 nm) (J. COHEN personnal communication) .

2°) Viral protein analysis

a) - Structural polypeptides

The electrophoregrams of the virus and the empty capsids showed two major populations of proteins (fig.2 a-b) with no significant contamination .

The capsid preparation was separated into two sharp peaks VP_1 and

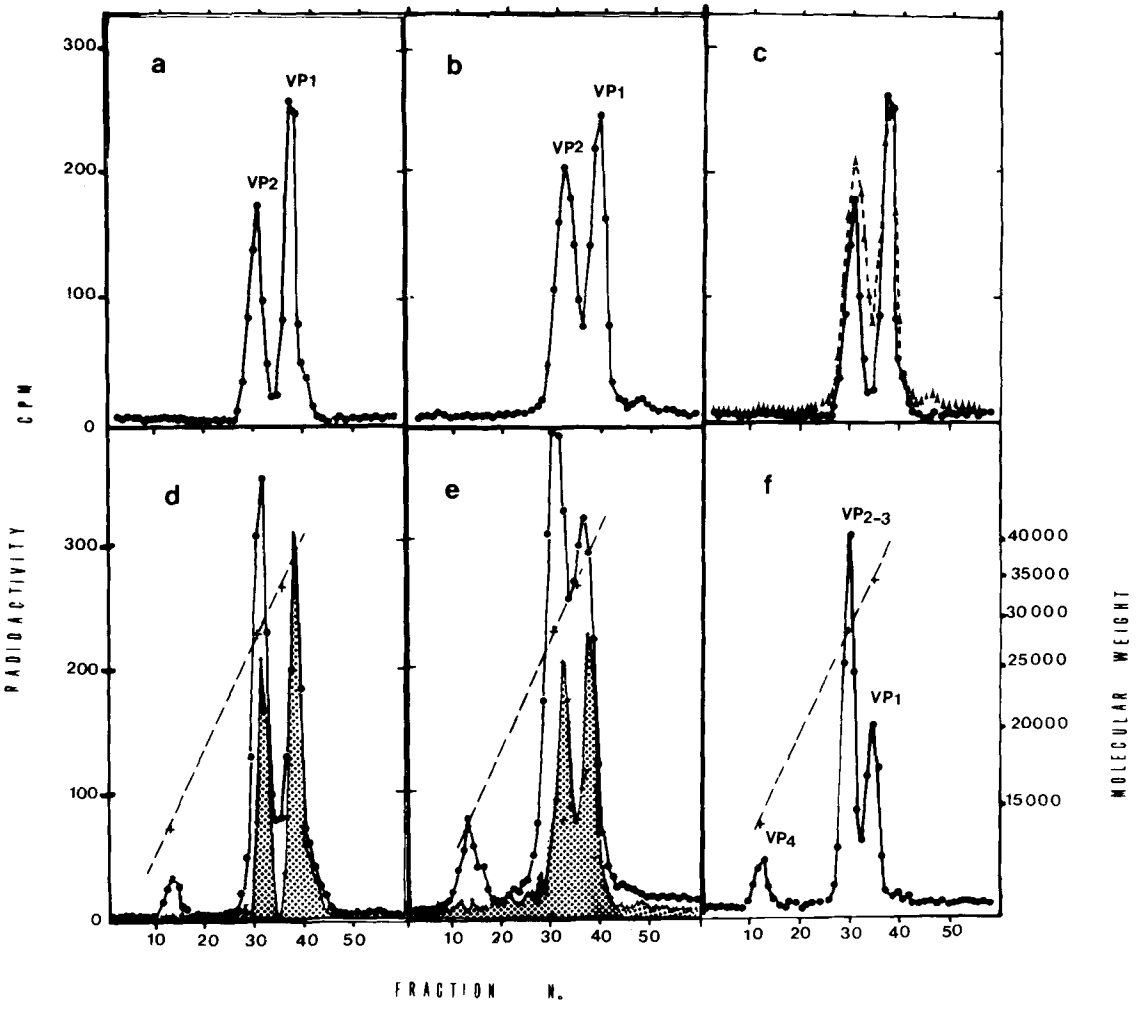

FIGURE 2
Electrophoresis of polypeptides from (14C) labelled viral proteins in 10% polyacrylamide gels, 0.I M sodium phosphate buffer pH 7,2 0,1 % S.D.S. migration was performed from right to left.
a) - empty capsids (b) complete particles (f) three fold purified F.M.D.V. O type .
c) - superimposed electrophoregrams O-----O empty capsids ▲-----▲ complete particles (d), (e) ; coelectrophoresis of empty capsids and complete particles with two fold purified F.M.D.V. Cross hatched peaks resulted from mathematical substraction of the values of F.M.D.V.

VP_2 (fig.2a) . However the viral preparation was not completely resolved and a little overlap was observed between the VP_1 and VP_2 peaks (fig.2b). When the two electrophoregrams of the empty capsids and the complete viral particles were superimposed in agreement with their "rf", the polypeptides,

VP_1 and VP_2 , seemed to have the same electrophoretic migration in polyacrylamide S.D.S. gel. (fig.2c) .

A major discrepancy was never the less seen : the aera ratio VP_1/VP_2 observed for the empty capsids was greater than that observed for the virions (1 as compare to 0,7).

To account for this difference two hypothesis were proposed :

- In the empty capsids there was less VP_2 than in the complete virion.
- In the complete virions there was one more protein ($VP_{2'}$) which could be the nucleoprotein, absent from the empty capsids preparation. The migration of which would take up an intermediate position between the VP_1 and VP_2 polypeptides .

b) - Molecular weight

As we hoped to differentiate this virus from the foot-and-mouth disease virus, we co-migrated the complete virion (fig.2b) and the empty capsids (fig.2a) with (14 C) amino acid labelled F.M.D.V. (fig.2b). The direct analysis of these electrophoregrams (fig.2 d-e) was uncertain so we undertake a mathematical treatment . We substracted all the values of foot-and-mouth disease virus profile (fig.2f) on the coelectrophoregrams. When we obtained the profile of the empty capsids (fig.2d crosshatched) and the S.V.D. virus (fig.2e crosshatched), each peak of F.M.D.V. was correctly positionned . This enabled us to draw a molecular weight diagram with the three proteins of this virus, having molecular weight of 34000, 28000 and 14000 (8-9) .

The VP_1 of the S.V.D. migrate further than the VP_1 of the F.M.D.V. The VP_1 protein of complete particles and empty capsids had a molecular weight between 37000 and 39000 .

The VP_2 migrate a little more than the VP_{2-3} of F.M.D.V. The molecular weight of this VP_2 was estimated between 29000 and 31000 .

As the empty capsid preparation was very pure, we could calculate the molar ratio of VP_1 and VP_2 (10). It was interesting to find that the two populations of molecules were in equimolar quantity .

If we applied this method to the complete viral preparation, we could see that for I.0 molecule of VP_1 there are I.3 to I.4 molecules of VP_2 . However as we could not eliminate 10 % to 20 % of the empty capsids in the viral preparation, this ratio must necessarily be greater .

Despite a similarity in pathogenicity of S.V.D. and F.M.D., the biochemical characteristics of these two viruses show major differences.

Particle size (30-32 nm) density (1,34 g/cm3), sedimentation coefficient (150 S) and molecular weight of the constitutive proteins enabled us to separate this new porcin enterovirus from F.M.D.V. : F.M.D.V. possessing a diameter of 23 to 25 nm, a density greater than 1,43 g/cm3 and a sedimentation coefficient (140 S) (5,11). The S.V.D.V. is more related to the enterovirus subgroup .

But the S.V.D. virus differed from the other picornaviruses in that no small detectible polypeptide, postulated to be associated with the viral RNA, could be demonstrated (11, 12, 13). Also this virus contains larger polypeptides (molecular weights 38000 and 30000) than the majority of the picornaviruses (MW 34000 to 25000) : enterovirus (12, 14, 15), cardioviruses (10, 13, 16, 17) rhinovirus and foot-and-mouth disease virus (9,11) . However some picornavirus posses larger polypeptides (42000 to 50000 MW for rhinovirus, and 30000 to 37000 MW for Echovirus) (18).

It has been established that the picornaviruses can produce both infective virus particles and empty capsids (procapsids) (19) . In polio and bovine enteroviruses (20) the procapsids which contain less polypeptide than the virus particle, are the precursor of virion particles, where a cleavage of the largest proteins occur during maturation (20-21) .

One of these new proteins posses the capacity to associate with the viral RNA (19) .

We are not able to demonstrate wether the empty capsids were products of viral breakdown or procapsids . No cleavage seemed to occure in the empty capsid proteins to give detectable shortened polypeptides having molecular weights less than 10.000 .

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