The Structural Components of Hog Cholera Virus

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Hog Cholera Virus, Structural Polypeptides, Viral RNA

Hog cholera virus grown in PK-15 cells and SK-cells was labeled with [^{35}S]methionine and [^{3}H]uridine. At least 3 polypeptides were resolved by polyacrylamide gel electrophoresis after disruption of the virus with sodium dodecylsulfate. The molecular weights of the structural proteins were determined to be 55000 (p55), 46000 (gp46), and 36000 (p36). The molecular weight of the viral RNA was determined to be about 4×10^6 in polyacrylamide-agarose-gel electrophoresis. In sucrose gradients the RNA has a $S_{20,w}$ value of 40–45S.

There is some evidence that Hog cholera virus belongs to the family Togaviridae which is proposed to include the four genera of Alpha-, Flavi-, Rubi-, and Pestiviruses [1]. For the final classification of Hog cholera virus it was necessary to study the viral structural components.

For determination of the protein composition of Hog cholera virus, PK-15 cells or SK-cells [2] were infected with Hog cholera virus strain ALD-970a [3] or strain PAV [4] respectively. After a 24 hour period of incubation, virus was labeled by adding [^{35}S]-methionine (10 μCi/ml, Buchler, Braunschweig) and actinomycin D (1 μg/ml, Serva, Heidelberg) to the culture medium which consisted of a mixture of VM3A [5] and Eagle's medium (10 : 1). The labeling period was for 12–16 hours. Uninfected cells were labeled similarly.

Labeling of viral RNA was accomplished by addition of [^{3}H]uridine (20 μCi/ml, Buchler) to the culture medium (VM3A + Eagle 10 : 1) at 24 hours after infection. Extracellular virus was harvested at 40 hours postinfection. The labeled virus was concentrated by sedimentation through a cushion of 20% (w/w) sucrose in TEN-buffer [6]. Further purification was achieved in a linear 20 to 50% (w/w) sucrose gradient in TEN-buffer. Electrophoresis of proteins in polyacrylamide gels was performed according to the methods described earlier [6]. Labeled Hog cholera virus ([^{35}S]methionine) concentrated and purified as described was precipitated with 5% trichloroacetic acid. The sediment was washed with ethanol and then redissolved in TEN-buffer containing 2% SDS. Reference proteins were usually co-electrophoresed with the radioactive viral proteins.

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After staining, the gels were cut into 1 mm slices which were prepared for scintillation counting by oxidizing with hydrogen peroxide. Insta-gel (Packard) was used as scintillation cocktail. Radioactivity was counted in a Packard Tricarb 3375.

The RNA of labeled ([^{3}H]uridine) Hog cholera virus was isolated as described by others [8]. Electrophoresis of the labeled RNA in polyacrylamide-agarose-gels was performed essentially according to the method of Loening [8] except that acrylamide (2.1%) was cross-linked with 0.12% bis-acrylamide. Gels were polymerized with 0.35% ammoniumpersulfate in glass tubes. Labelled viral RNA was co-electrophoresed with unlabeled cellular 28S and 18S RNA. After staining of the gels the positions of the reference bands were localized and the gels were cut into 1 mm slices and prepared for scintillation counting as described above.

In both, virus from PK 15- and SK-cells, the main polypeptides resolved by polyacrylamide gel electrophoresis had molecular weights of 54000–56000, 45000–47000, and 35000–37000 Dalton (Fig. 1 A and B). Another polypeptide which was seen occasionally had a molecular weight of about 65000. It appeared that this latter protein was an uncleaved precursor of one of the structural proteins since it was only sometimes incorporated into the virion. Two or three additional polypeptides were found occasionally with molecular weights in the range of about 30000–35000 and 18000–25000 Dalton. We assume that these polypeptides are degradation products which arise from proteolytic cleavage because they occurred mainly in older preparations of Hog cholera virus. In the supernatant of mock-infected PK-15 cells viral polypeptides were never found (Fig. 1 A). Hog cholera virus grown in prelabeled [7] PK-15 cells contained no significant amount of the radioactive label (Fig. 1 A).

The radioactivity-profile obtained after electrophoresis of viral RNA is shown in Fig. 2 A. Estimations of the molecular weight using the migration distances of ribosomal RNAs ranged between 3.8 and 4.2×10^6. A radioactivity-profile of a gel after electrophoresis of whole RNA isolated from infected PK-15 cells is shown in Fig. 2 B. In addition to the cellular marker-RNAs, a peak of radioactivity with the same migration mobility as the viral RNA (Fig. 2 A) was observed (Fig. 2 B). This latter RNA was resistant to the action of actinomycin D as is shown in Fig. 2 C and is, therefore, expected to be the RNA of Hog cholera virus. In a linear 10–30% (w/w) sucrose gradient labeled RNA isolated from virus particles revealed a sedimentation coefficient of 40–45S (Fig. 3).

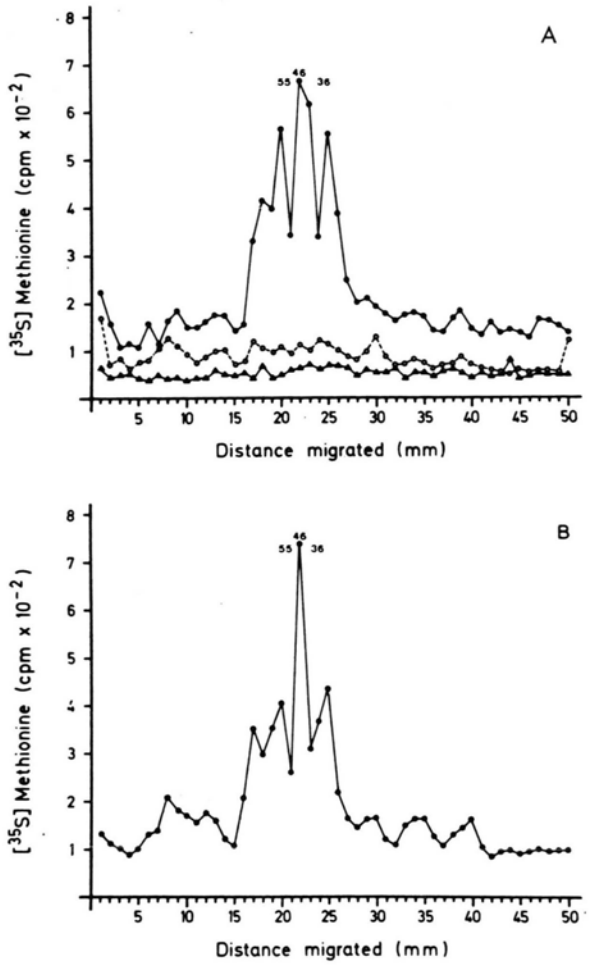

Fig. 1. SDS-PAGE of Hog cholera viral proteins. A) Structural proteins of Hog cholera virus strain ALD-970a grown in PK-15 cells (●—●); Radioactivity profiles obtained from the supernatant of mock-infected (▲—▲) and prelabeled PK-15 cells (○—○). Prelabeled cells were infected after a labeling period of 24 h. Extracellular virus was harvested 24 hours post infection. B) Structural proteins of Hog cholera virus strain PAV grown in SK-cells (●—●). The molecular weights of the viral proteins were estimated by comparing their electrophoretic mobility relative to marker proteins (bovine serum albumin, gammaglobulin H-chain, ovalbumin, and chymotrypsinogen) run in the same gel. The polypeptides were identified in the following fractions: p55 in fraction 20, gp46 in fraction 22, p36 in fraction 25. The polypeptide in fraction 17 appeared only occasionally in the virion and is thought to be an uncleaved precursor of one of the 3 other structural proteins.

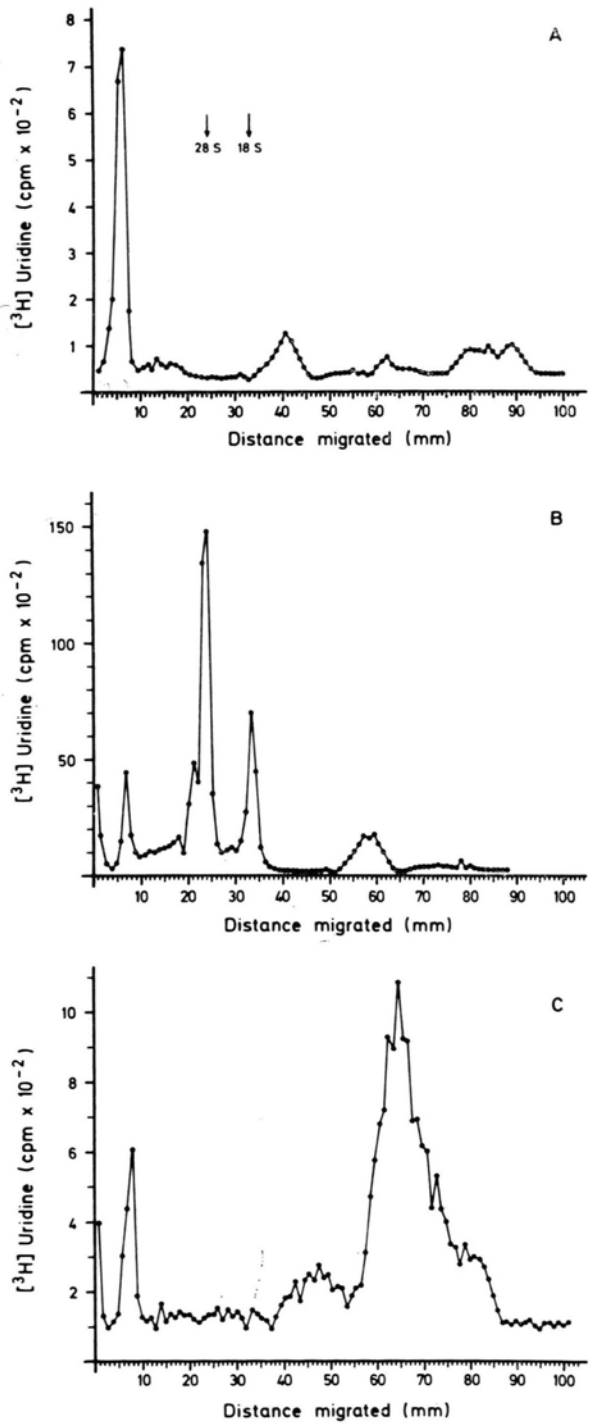

Fig. 2. Electrophoresis of Hog cholera viral RNA. A) Viral RNA was subjected to co-electrophoresis with unlabeled ribosomal RNA. Positions of the 28S and 18S RNA markers are indicated by arrows. The viral RNA was found in fraction 6. B) Cytoplasmic RNA extracted from infected PK-15 cells. C) Cytoplasmic RNA extracted from infected PK-15 cells treated with actinomycin D (1 μg/ml).

Based on the susceptibility to lipid solvents [9] and on electron microscopic studies [10] Hog cholera virus was considered to be a member of the Togavirus family. We have presented additional evidence for the classification of this virus as a Togavirus since the polypeptide pattern of the virion is similar to

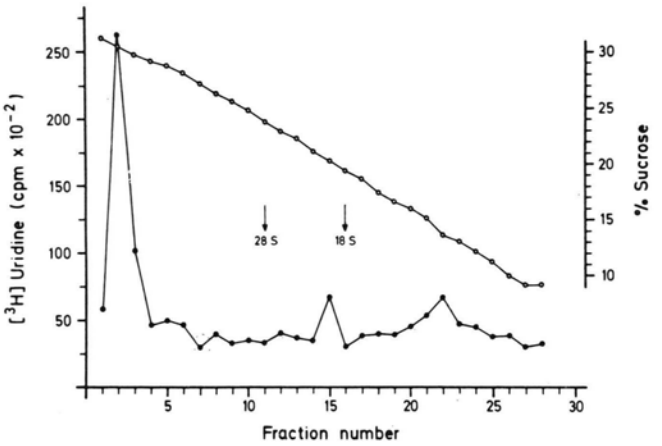

Fig. 3. Velocity sedimentation of Hog cholera virus RNA. RNA extracted from Hog cholera virus was sedimented in a linear 10–30% (w/w) sucrose gradient (○–○). The gradient was centrifuged for 10 hours at 25000 rpm in a SW 25 rotor (Beckman, Spinco). Arrows indicate the positions of 28S and 18S marker RNA.

that of alphaviruses which are composed of two glycoproteins in the viral membrane and one core protein with molecular weights within the range of 50000 and 30000, respectively. A further glycoprotein found in Semliki Forest virus has a molecular weight of about 10000 Dalton [11]. Hog cholera virus is composed of a least 3 polypeptides: p55, gp46, and p36 (Fig. 1). By staining with the periodic acid-Schiff reagent [6] it was shown that the 46000 Dalton polypeptide contained carbohydrate chains (result not shown). The molecular weight of the viral RNA was found to be about 4.0×10^6 (Fig. 2). In sucrose gradient the viral RNA revealed a sedimentation coefficient of 40–45S (Fig. 3). By comparing Hog cholera virus RNA and Sindbis virus RNA it was noted that the sedimentation coefficient of Sindbis virus RNA was slightly lower than that of Hog cholera virus RNA. In contrast the molecular weight of Sindbis virus RNA determined in composite polyacrylamide-agarose-gel electrophoresis was slightly higher than that of Hog cholera virus RNA (results not shown). Therefore the RNAs of these viruses may differ in their secondary structure.

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