Capsid Components of the Parvovirus H-1[1] (36329)

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H-1 virus is a small (200–220Å) DNA-containing virus belonging to the parvovirus group (1). Other representative members of this group are Kilham's rat virus (RV) (2), the minute virus of mice (MVM) (3), and the adeno-associated viruses (AAV) (4, 5). All of these agents are defective in some cell systems though only the AAV are completely dependent on their helper adenoviruses. H-1 and RV share the ability to produce a characteristic deformity in newborn hamsters; they are, however, serologically distinct and show no cross neutralization (6).

Recent evidence shows that the DNA of H-1 and RV is single-stranded (7, 8), with a molecular weight of about 1.7×10^6 (8, 9). The amount of information specified by a genome of this size is therefore limited, probably on the order of 4–6 proteins. Salzman and White (10), working with RV, and Rose *et al.* (personal communication), working with AAV, report that both RV and AAV contain one major and two minor protein components.

This study shows that H-1 is also composed of three electrophoretically-distinct proteins, the relative amounts of which are similar to those reported for Kilham's rat virus (10). In addition we have determined the amino acid composition of the intact H-1 virion.

Materials and Methods. Cell culture and virus propagation. The methods of cell culture, virus propagation and purification have been previously described (11). In our present studies we have employed hamster embryo cultures only for virus propagation as greater yields of "full" (DNA-containing) virions were obtained than in Salk "monkey heart" or human NB kidney cell cultures where the yield of empty protein coats, particularly in the former cells, exceeded that of the infectious particles.

Preparation of radioactive virus. Cell cultures in Hanks' minimal essential medium (HMEM) were infected at less than 1 PFU/cell and adsorption was allowed to proceed for 1.5 hr at 37°. The medium was then removed and replaced with medium containing 1/20 the normal amount of amino acids in 5% dialyzed fetal bovine serum. ^{14}C- or ^{3}H-labeled protein hydrolysate (1 ml at 1 mCi/ml) was diluted to 22 ml with medium and 0.5 ml was added to each of 44 infected cultures. Virus was grown in the presence of radioactive amino acids for 60 hr at 37°. Radioactive H-1 was then purified by a recently developed detergent method (Rhode personal communication). A 10^{-8} dilution of nonradioactive H-1 prepared by this method killed 2 litters of newborn hamsters in 6 days.

Solubilization of H-1 virus proteins. One volume of CsCl-banded H-1 (0.2–0.4 ml) was mixed with an equal volume of 0.01 *M* sodium phosphate, pH 7.2, containing 4% sodium dodecylsulfate (SDS) and 2% mercaptoethanol (ME) (12). The mixture was then placed in a water bath at 100° for 1 min.

Polyacrylamide disc gel electrophoresis. Gels were prepared according to Summers *et al.* (13). The solubilized virus proteins were mixed with 1/4 vol of 40% sucrose containing 1% bromophenol blue and layered on 7.5% gels. Runs were made at 26° using 5 mA/-tube until the tracking dye reached the bottom of the gels (4–5 hr on 6.5 cm gels or 16 hr on 12 cm gels). The tray buffer was 0.1 *M* sodium phosphate, pH 7.2, containing 0.1% SDS. Reference proteins used as molecular weight markers were freshly prepared in 0.01

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M phosphate, pH 7.2, containing 1% SDS and 1% ME and incubated at 37° for 2 hr before use. Virus polypeptides and protein markers (serum albumin, ovalbumin, pepsin, ribonuclease A, and deoxyribonuclease I (20 μg each) were run in the same or parallel gels. Gels to be stained were fixed in 10% trichloroacetic acid (TCA), stained with 0.1% Coomassie blue in 50% methanol-7.5% acetic acid (12), and destained with 7% acetic acid containing 20% ethanol.

Radioactive gels were frozen at —70° and sliced into eighty 1 mm discs. Radioactive proteins were eluted overnight at 50° with 30% H_2O_2 (0.1 ml), solubilized with 1 ml of NCS (Amersham-Searle), and counted in a toluene-spectrafluor scintillation mixture (14) with a Mark I Nuclear-Chicago analyzer.

Elution of protein components from gels. Samples were run in quadruplicate, with the first gel used for staining and destaining to locate the bands (15). Corresponding areas on the unstained gels were cut out, macerated and extracted with 0.1% SDS at 37° for 3 hr. After two successive extractions, the eluants were combined, filtered through 0.45 μ Millipore filters and dialyzed against 0.05 *M* Tris containing 0.1 *M* NaCl and 0.001 *M* EDTA (pH 7.4). The protein fractions were then lyophilized, dissolved in distilled H_2O, and tested for hemagglutination activity against guinea pig red blood cells (6).

Amino acid analyses. Virus from several purifications was pooled, concentrated by ultrafiltration and dialyzed against distilled H_2O. Samples were lyophilized in weighed vacuum hydrolysis tubes and 1 ml of 6N HCl was added. After freezing and evacuation, the virus was hydrolyzed for 24 hr at 110°. The hydrolysate was evaporated to dryness, dissolved in pH 2.2 sample buffer, filtered through 0.45 μ Millipore filters, and run on a Beckman Model 120C analyzer equipped with a digital integrator. The data were analyzed on an Olivetti 101 computer by comparison with a standard amino acid mixture.

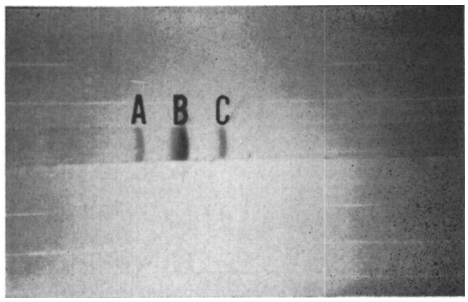

FIG. 1. Proteins of H-1 virions: CsCl-banded H-1 was disrupted by heat and SDS and separated by electrophoresis as described in Methods. The protein fractions were stained with Coomassie brilliant blue. Migration is toward the anode at the right.

Results and Discussion. Figure 1 shows the electrophoretic separation of SDS-dissociated H-1 virions on 7.5% acrylamide gels. Three components, labeled A, B, and C, migrated toward the anode. When radioactive

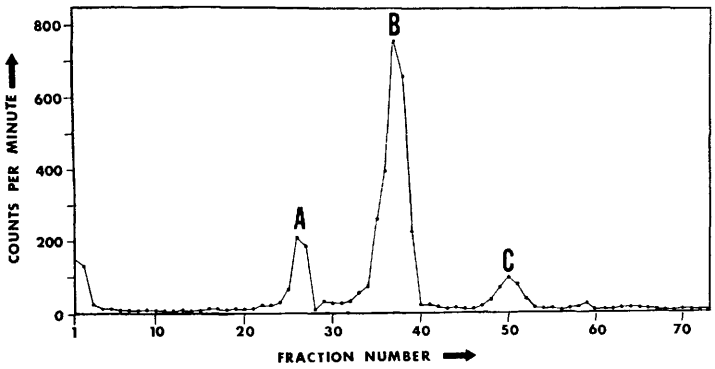

FIG. 2. Distribution of radioactive H-1 proteins on acrylamide gels: Radioactive ^{14}C-H-1 proteins were separated on 7.5% gels. The gels were frozen, sliced into eighty 1 mm discs and the radioactivity was measured as outlined in Methods. Migration is toward the anode at the right.

TABLE I. Proteins of H-1 Virus.

Gel component	Mol wt[a] ($\times 10^{-3}$)	Percentage of each radioactive component[b]	Approx no. of molecules/virion[c]
A	92	15	8
B	72	75	52
C	56	10	9

[a] Estimations are based on the migration of ^{14}C-radioactive virus proteins relative to that of molecular weight markers on 7.5% SDS-acrylamide gels (16).

[b] Distribution of radioactivity in each protein component relative to total number of counts of viral protein applied to gels.

[c] Calculations based on 4.95×10^6 daltons of total protein per virion.

^{14}C-labeled H-1 was disrupted and similarly separated on acrylamide gels, the distribution of radioactivity representing components A, B, and C was 15%, 75% and 10%, respectively (Fig. 2). Ninety percent of the radioactivity applied to the gels was recovered. Coelectrophoresis of the viral proteins with reference protein markers allowed an estimate to be made of the molecular weight of the viral polypeptides (16). Electrophoretic separation of H-1 capsid components in the same or parallel gels with bovine serum albumin [67,000], ovalbumin [43,000], pepsin [35,000], ribonuclease A [13,700], and deoxyribonuclease I [31,000] gave average ($\pm$ 10%) molecular weight estimates for components A, B, and C of 92,000, 72,000, and 56,000 daltons, respectively. Knowing the distribution of radioactivity for each of the components, their molecular weights and the total daltons of virus protein, the total number of A, B, and C molecules per virion can be calculated. The molecular weight of H-1 has been estimated at 6.6×10^6 daltons, of which 75% or 4.95×10^6 daltons is protein (8). Each virion would contain, therefore, about 8 molecules of component A; 52 molecules of B and 9 molecules of C (Table I). Thus there are about 6 molecules of B for every molecule of A and C present.

Morphologically, H-1 appears to be an icosahedron with 32 capsomers, *i.e.*, 12 pentamers and 20 hexamers (17). Assuming H-1 contains 32 capsomers, our major component B would represent the main structural protein. Furthermore, elution of components A, B, and C from acrylamide gels and assay of their hemagglutinating activity showed that B gave a weak but distinct reaction (1:80), while A and C components failed to react.

Components A and C which account for only 15% and 10% of the total H-1 protein, respectively, may represent internal constituents of the virions. They appear on acrylamide gels of both SDS-dissociated virions and empty capsids. Their association with the DNA of the infectious particles is, therefore, unlikely. Polyoma virus, on the other hand, contains internal, basic polypeptides apparently associated with the DNA (18). Fractionation of detergent-dissociated H-1 on pH 4.3-urea gels failed to reveal any components migrating toward the cathode. It is interesting that despite the size difference between H-1 (220Å) and RV (180Å) (17) and their serological unrelatedness, both H-1 and RV virions contain the same relative amounts of A, B, and C components (10). The total mol wt of H-1 proteins [220,000 (Table I) is about 16% greater than that for RV (189,000)] (10).

The amino acid content of the H-1 protein coat (Table II) indicates that the proteins are rich in aspartic and glutamic acids, with an average acidic/basic amino acid ratio of 1.75. The high content of glycine is probably the result of purine breakdown in the H-1 DNA (19). In a personal communication, Rose, Maizel, and Shatkin, report that another parvovirus, AAV, is also rich in acidic amino acids (acidic/basic amino acids = 2.35). It may also be noteworthy that the protein coat of another small DNA virus, the phage $\phi \times 174$, has an acidic/basic amino acid ratio similar to that of H-1 (1.70) (20).

Summary. Disruption of purified H-1 virions with sodium dodecyl sulfate (SDS) and mercaptoethanol (ME) at 100° and pH 7.2 results in the appearance of one major and two minor migrating components on polyacrylamide gels. Molecular weights of 92,000 (A), 72,000 (B), and 56,000 (C) were determined for the three moieties by coelec-

TABLE II. Amino Acid Composition of H-1 Capsid Protein.[a]

Amino acids[c]	Expt.[b] 1	2	Av
Lysine	5.95	7.09	6.52
Histidine	3.55	2.49	3.02
Arginine	3.31	3.00	3.15
Aspartic acid	9.76	11.78	10.77
Threonine	6.23	7.35	6.79
Serine	5.53	8.44	6.98
Glutamic acid	11.92	11.07	11.49
Proline	7.51	7.92	7.71
Glycine	13.03	12.89	12.96
Alanine	8.02	8.04	8.03
Cysteine	Trace	Trace	Trace
Valine	5.29	4.23	4.76
Methionine	2.37	1.76	2.06
Isoleucine	4.18	3.48	3.83
Leucine	8.63	7.05	7.84
Tyrosine	1.46	0.45	0.95
Phenylalanine	3.20	2.96	3.08
Acidic/basic	1.69	1.81	1.75

[a] Data expressed as mole percentage of total moles of amino acids recovered.

[b] Determinations were made on two separate virus preparations.

[c] No corrections were made for hydrolytic losses.

trophoresis of the dissociated virus with reference proteins of known molecular weight. Electrophoretic separation of ^{14}C- or ^{3}H-labeled virus polypeptides showed the relative amount of each radioactive component to be 15% (A), 75% (B) and 10% (C) of the total viral protein. Elution of the components from the gels, followed by hemagglutination assay against guinea pig red blood cells, indicated that component B was the antigenic component and structural protein. Amino acid analyses of H-1 virions revealed an overall acidic nature of the protein (acidic/basic amino acids = 1.75).

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