Architecture of the Adenovirus Capsid

KENDALL O. SMITH, WARREN D. GEHLE, AND MELVIN D. TROUSDALE

Department of Virology and Epidemiology, Baylor University College of Medicine, Houston, Texas

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ABSTRACT

SMITH, KENDALL O. (Baylor University College of Medicine, Houston, Tex.), WARREN D. GEHLE, AND MELVIN D. TROUSDALE. Architecture of the adenovirus capsid. *J. Bacteriol.* **90**:254-261. 1965.—The capsids of adenovirus type 2 were fragmented by treatment with low concentrations of sodium lauryl sulfate. The clusters of capsomeres resulting from this treatment displayed characteristic patterns. Some of these clusters, each consisting of nine capsomeres, interlocked so as to form the triangular facets of the viral icosahedron. There is some evidence which suggests that the capsomeres are connected to each other by filamentous structures located near their bases. Connections between capsomeres along the edges and at the vertices of the triangular facets were the first to break when particles were treated with sodium lauryl sulfate. Further treatment broke connections between other capsomeres. These data provide additional information concerning the capsomere arrangements and the fine structure of adenoviruses.

The application of negative staining to the study of virus fine structure has led to some fruitful studies of their construction (Brenner and Horne, 1959; Horne et al., 1959; Horne and Wildy, 1962; Wilcox, Ginsberg, and Anderson, 1963; Mayor, 1964). Horne et al. (1959) found that adenoviruses were 72 m μ in diameter, icosahedral in shape, and possessed 252 morphological subunits (capsomeres) on their surface. In the course of our studies of tumorigenic adenoviruses, we applied various methods for degrading whole particles into smaller components (Smith, 1965 and *J. Immunol.*, *in press*). One method, sodium lauryl sulfate (SLS) treatment, released circular deoxyribonucleic acid (DNA) strands (Smith, 1965) and degraded the virus protein shell (capsid) into fragments consisting of characteristic subunit patterns. This report describes those fragments and suggests the manner in which they probably fit together in the intact virus capsid.

MATERIALS AND METHODS

Adenovirus strains. The following strains were used: type 2 (Benyesh-Melnick), type 7 (Ginsberg), type 12 (Huebner), and type 18 (Huebner). All were verified as to type by serum neutralization and by an agglutination reaction recently described (Smith, Trousdale, and Gehle, *J. Immunol.*, *in press*).

Cell cultures. Human epithelial cells (strain KB, obtained from Maurice Green, Washington University) were used for routine passage and growth of virus (37 C). Spinner cultures (500 to 1000 ml each) were usually employed for virus production. Eagle's medium with 10% fetal bovine

serum was used as nutrient fluid for growth of cells and for maintenance of infected cells.

Purification and concentration of viruses. A cesium chloride density-gradient fractionation method was used (Smith, 1965 and *in press*). Only the lowest (density 1.34 to 1.36) bands of virus were used for this study. Adenovirus particles of this density were usually the most perfect structurally (Smith, *in press*).

Treatment with SLS. SLS (obtained from Nutritional Biochemicals Corp., Cleveland, Ohio) was dissolved in 0.15 M sterile saline to give concentrations ranging from 30 to 500 μ g/ml. SLS solution was added in equal volumes to purified virus, and the mixtures were allowed to incubate at room temperature for 5 to 60 min. The concentrations of SLS given in the Results section were final concentrations in the virus-SLS mixtures.

Electron microscopy. The preparative and staining procedures used have been described before (Smith and Melnick, 1962; Smith, 1965 and *in press*). Uranyl acetate was used in higher concentrations (0.5 to 1%) in such a way as to give a negative staining effect. Potassium phosphotungstate, although effective as a negative stain for adenoviruses, was found to be less suitable than uranyl acetate for revealing the fine structure of adenovirus capsomeres.

RESULTS

Figure 1 illustrates the appearance of purified adenovirus type 2 particles when negatively stained. Interpretation of the capsomere arrangements and the fine structure of each capsomere on the surface of whole viruses is not always easy, because the images of both sides of a particle are

FIG. 1. Purified adenovirus type 2 particles (the bar in this and subsequent micrographs equals 100 μ). The model shown in the right corner shows the same design as that described by Horne et al. (1959).

superimposed. However, occasional fortuitous combinations of staining and particle orientation make it possible to resolve parts of the orderly subunit arrangement suggested in the model constructed by Horne et al. (1959; Fig. 1, lower right).

The treatment of purified adenovirus type 2 with low concentrations of SLS (30 μ g/ml) resulted in a very slight degradation of capsids (Fig. 2). The first indication of capsid disruption was a slight change in the appearance of the particle, involving only one or two triangular facets (see arrows). Fragments composed of nine capsomeres were occasionally observed (see Fig. 2, left). These capsomeres showed remarkable uniformity in arrangement. Greater concentrations (60 to 120 μ g) of SLS caused more obvious particle disruption and the release of numerous clusters of nine capsomeres (Fig. 3). The typical pattern of these clusters is shown in the insert in the lower right corner of Fig. 3. Such groups (hereafter referred to as type A groups) consisted of six subunits in the form of a triangle, with one capsomere attached in a characteristic position to each side of the triangle.

Lower magnification electron micrographs of these subunits are shown in Fig. 4. Couples of

connected type A groups are indicated by the arrows. The diagram inserted in the lower right corner of Fig. 4 indicates the arrangement of capsomeres within the couples (hereafter referred to as type B groups). Occasionally as many as 19 type A groups could be seen localized in one area on a prepared grid, although it was apparent that one or two capsomeres were sometimes missing from individual groups (see lower micrograph, Fig. 4).

Occasionally it was possible to find type A groups connected to each other so as to form units of three and four. Figure 5 illustrates the appearance of two such groups in one field. The group A pattern is well preserved in these groups of three and four, which are joined in the same manner as that seen in the type B groups. The three capsomeres located on the three sides of each triangle interlock with neighboring type A groups in a design which suggested the model shown in the upper right corner of Fig. 5. These arrangements conform to those shown in the model of Horne et al. (Fig. 1).

Rarely, we found starlike arrangements of five type A groups connected to each other and arranged in a circle (Fig. 6). Not every capsomere in the five type A groups could be seen clearly in

FIG. 2. Purified adenovirus type 7 particles after treatment with 30 μ g/ml of sodium lauryl sulfate (SLS). Black arrows point to clusters of capsomeres. Other arrows indicate points of change on the virus particle.

a single micrograph, but enough were visible in micrographs such as the one shown in Fig. 6, left, to lend credence to the model shown at the bottom of Fig. 6. In each case where five type A groups were joined in this fashion, most of the six subunits which would fit into the center were missing (see dots). One of these six capsomeres is present in the group shown on the left of Fig. 6, however. The group on the right, though it shows little detail of subunit arrangement, has almost the exact size, symmetry, and outline as the group on the left and that represented in the model. Type A and B groups were common in most SLS-disrupted virus preparations, but units of three, four, and five type A groups were progressively more difficult to find.

The size of the capsomere groups produced seemed to be dependent upon the concentration of SLS used. Many type A groups were produced by 120 μ g of SLS/ml, but the addition of more SLS to such a treated suspension resulted in fragmentation of type A groups to smaller clusters of capsomeres. Fragmentation of capsomeres by a given amount of SLS appeared to be complete within 15 min after mixing.

A unit of six capsomeres would provide the

"missing piece" at the center of every five type A groups and would form the point at which five triangular facets meet (see dots, Fig. 6). In spite of much searching, no such six-capsomere arrangements were ever found in SLS-treated preparations. Only smaller groups of one, two, and three capsomeres were encountered. It is probable, therefore, that the groups of one, two, and three capsomeres found with type A and B groups represent the fragments of the six-capsomere "missing piece" indicated in Fig. 6.

We attempted to dismember the particle while it was fixed in place on the surface of agar, so as to limit the dispersion of components from individual particles. Agar (2%) blocks of 2- to 3-mm thickness were placed on wire screening and lowered into dishes containing SLS so as to barely wet the lower surface of the agar. Small droplets of purified virus were then placed on the top surfaces of the agar. The droplets containing the virus dialyzed into the agar (leaving the particles on the upper agar surface), and the SLS dialyzed through the agar from the bottom to disrupt the particles. The components were recovered in the usual way by pseudoreplication. The appearance of particles disrupted in this

FIG. 3: Purified adenovirus type 2 after treatment with SLS. Type "A" groups of capsomeres are arranged in patterns which suggested the design shown in the lower right corner.

manner is illustrated by the micrograph in Fig. 7. Capsomeres can be seen in profile (side view) in several places. It appears that the capsomeres are connected to each other by structures located near their bases (see arrows near center of Fig. 7). Short filaments measuring about 78 Å in length

project from these bases, which measure about 67 Å in width. Type A groups can be seen in the lower right of Fig. 7. The details of capsomere fine structure and the patterns by which they are connected are being studied and will be presented later.

FIG. 4. *Purified adenovirus type 2 after treatment with SLS. Note the large number of type "A" groups; connected type "A" groups formed the pattern shown in the lower right corner.*

FIG. 5. Purified adenovirus type 2 after treatment with SLS. Type "A" groups are arranged in a pattern suggesting the design shown in the upper right corner.

FIG. 6. Purified adenovirus type 2 after treatment with SLS. Type "A" groups are arranged so as to suggest the design shown in the lower part of the figure.

FIG. 7. Purified adenovirus type 2 after treatment with SLS. Capsomeres are seen in profile (see arrows) and as usual (lower right).

All of the micrographs shown above except the one in Fig. 2 were obtained from preparations of adenovirus type 2. Adenoviruses types 7, 12, and 18 were treated similarly and were found to yield type A and B groups readily. Thus, these four adenovirus types fragment in the same way when treated with SLS and are probably constructed in a similar, if not identical fashion.

DISCUSSION

The chemical mechanism by which SLS breaks bonds between adenovirus subunits in the definite pattern described in this report is not clear. SLS, being an anionic detergent, would be expected to act upon hydrophobic moieties. At least two possible mechanisms might account for the fragmentation of adenovirus capsids into characteristic patterns of capsomeres: (i) selective breakage of some bonds between capsomeres, leaving other bonds unaffected (this suggests qualitative differences between bonds); or (ii) certain groups of capsomeres are linked together by different numbers of the same kind of bond. In the latter case, fragmentation of a particle into characteristic groups of capsomeres would reflect the probability of breaking all bonds between some capsomeres while leaving others connected. The fact that type A and B groups were further fragmented by

treating with higher concentrations of SLS strongly suggests that the latter mechanism is involved. The points of weakest bonding between adenovirus capsomeres appear to be along the edges and at the vertices of the triangular facets.

Whether type A group capsomeres are antigenically different from those forming the vertices of the virus particle remains to be determined. Perhaps it will be possible to resolve this question by visualizing the attachment of type- and group-specific antibody molecules to these distinctive capsomere groups, by use of negative staining methods (Almeida, Cinader, and Howatson, 1963; Elek, Kingsley Smith, and Highman, 1964; Smith et al., *in preparation*). The six-capsomere "missing piece" has about the same size (20 μ diameter) and structure as small particles recently found to be associated with certain adenovirus preparations (Melnick et al., 1965). Mayor et al. (1965) showed that these particles contain DNA and have a buoyant density of 1.43. We have no direct evidence, however, that the 20- μ particle is a structural component of adenoviruses we have studied.

Type A groups, which consist of nine capsomeres each, probably form the faces of the 20 triangular facets making the adenovirus icosah-

hedron, and thus account for 180 subunits. The six-capsomere groups which form the 12 vertices displaying fivefold symmetry account for 72 additional subunits (Fig. 5). The total, 252, is the number of capsomeres proposed for adenoviruses by Horne et al. (1959). The arrangements we observed in partially degraded particles are in agreement with their model.

The data presented in this paper provide new information concerning the subunit arrangements in the adenovirus capsid and the manner in which the subunits are held together. Controlled degradation of virus particles and examination of the fragments should be helpful in further understanding the architecture of the virion.

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