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Ion Etching of Human Adenovirus 2: Structure of the Core

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The surface of human adenovirus 2 was etched by irradiating intact virions with low-energy (1-keV) Ar^+ ions in a Technics Hummer V sputter coater. Viral structures exposed by the etching process were shadowed and then examined in the electron microscope. Periods of etching that were sufficient to reduce the viral diameter by 20 to 30 nm revealed distinct substructural elements in the virion core. Cores were found to consist of a cluster of 12 large, uniformly sized spheres which abutted one another in the intact virion. The spheres, for which we suggest the name "adenosomes," had a diameter of 23.0 ± 2.3 nm, and they were related to each other by two-, three-, and fivefold axes of rotational symmetry. The results support the view, originally suggested by Brown et al. (J. Virol. 16:366–387, 1975), that the adenovirus 2 core is composed of 12 large spheres packed tightly together in such a way that each is directed toward the vertex of an icosahedron. Such a structure, constructed of 23.0-nm-dimater spheres, would have an outside diameter (vertex-to-vertex distance) of 67.0 nm and a face-to-face distance of 58.2 nm. It could be accommodated inside the icosahedral adenovirus capsid if each large sphere were located beneath a capsid vertex.

Convincing studies have now shown that adenovirus particles consist of an icosahedral capsid surrounding a DNA-containing core (5, 14, 19). The details of the capsid architecture are well known (7), but considerable uncertainty exists about the structure of the core. When examined in an electron microscope, thin sections of fixed adenovirus virions show that the core is hexagonal in overall shape and that it contains the viral DNA (4). Cores can be isolated preparatively by extracting whole virions with several different agents, including deoxycholate (13, 16, 17), acetone (8), Sarkosyl (2), 10% pyridine (15), and others (19). Studies with isolated cores show that they are compact structures with a diameter of 64 to 68 nm and a density of 1.42 g/cm^3. Cores prepared by Sarkosyl extraction contain the viral DNA plus protein VII, whereas those isolated by other methods contain protein V as well (2, 8, 9). Electron microscopic examination of Sarkosyl cores showed them to be loose aggregates of 8 to 14 large spheres whose average diameter was 21.6 nm (2). Cores prepared by other methods are found to be condensed, roughly spherical objects, but in most cases they lack clearly defined substructural features (8, 13).

Two major models, the "vertexful" model and the "nucleosome" model, have been proposed to describe the structure of the adenovirus core. The vertexful model is based on the observed structure of Sarkosyl cores. It is proposed that the core consists of 12 large spheres, each with the diameter (21.6 nm) observed in Sarkosyl cores. Each large sphere is considered to be located at a vertex of an icosahedron which, in the intact virus, would lie just below a vertex of the capsid icosahedron (2).

The nucleosome model is quite different. According to this proposal, the basic structural element of the core is a nucleosome-like particle ca. 10 nm in diameter. Each nucleosome would consist of six molecules of virion polypeptide VII, one molecule of protein V, and 150 to 200 base pairs of DNA. A total of 180 such nucleosomes, which would account for all the viral DNA, would be condensed inside the adenovirus capsid (3). This model is consistent with the condensed but featureless appearance of most cores. It is supported by the results of experiments in which permeabilized virions are treated with micrococcal nuclease (3, 11, 18). In those studies the viral DNA was digested to a limiting size of 150 to 200 base pairs, which is considered to be the amount of DNA protected by each nucleosome.

The studies described here were undertaken to test the idea that ion etching methods might be employed to resolve the uncertainty about the structure of the adenovirus core. Ion etching, as first adapted for viral studies by Bendet and Rizk (1), involves the use of low-energy ion beams, or plasmas, to erode virus surfaces. It depends on the fact that N_2^+ or Ar^+ beams of 1 to 5 keV can break covalent chemical

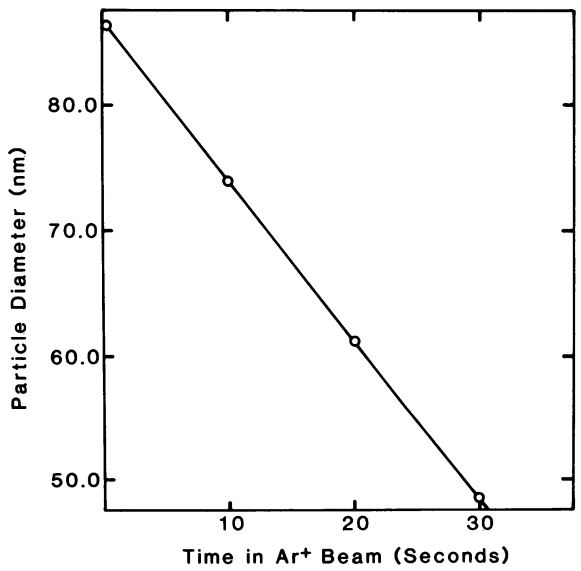

FIG. 1. Effect of Ar^+ etching on the apparent diameter of adenovirus 2. Intact adenovirus 2 virions were etched for various times with Ar^+, directionally shadowed, and examined in an electron microscope as described in the text. Particle diameters (vertex-to-vertex distances) were measured from fivefold enlargements of electron microscope negatives. Points shown are average values obtained from at least 10 different particles measured in representative electron microscope fields.

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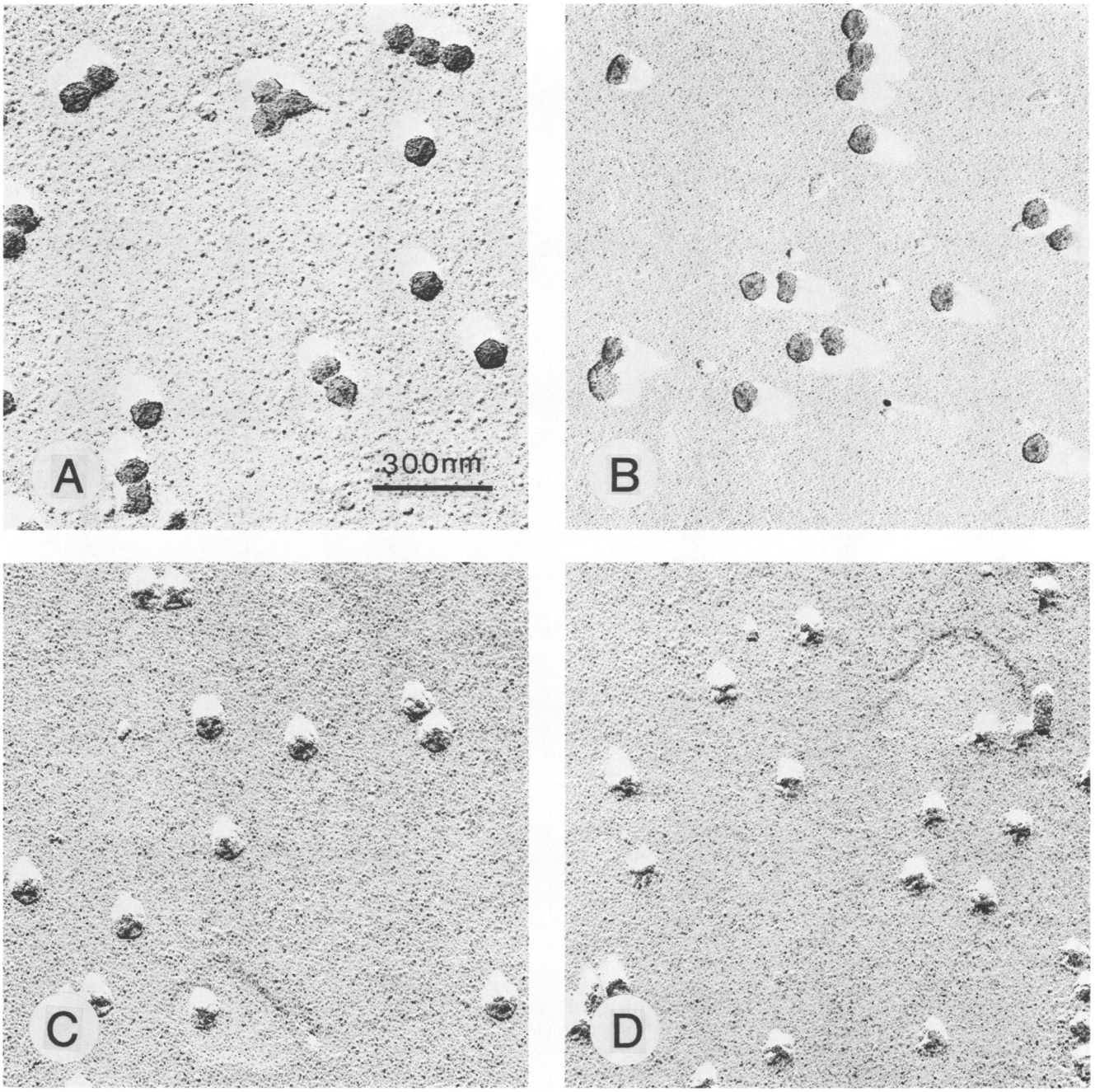

FIG. 2. Morphology of adenovirus 2 after etching with Ar^+. Etching of intact virions, directional shadowing, and electron microscopy were carried out as described in the text. Images show unetched virions (A) and virions etched for 10 s (B), 20 s (C), and 30 s (D).

bonds but are not energetic enough to penetrate deeply into the interior of virus particles. Irradiation of intact viruses, therefore, should produce a gradual erosion of the particle surface and expose internal components in a reasonably undisturbed state. In the experiments reported below, low-energy Ar^+ ions were employed to erode the surfaces of adenovirus 2 particles. Etched virions were then examined in an electron microscope for the presence of exposed core structures.

MATERIALS AND METHODS

Adenovirus 2 growth and purification. Human adenovirus 2 (obtained from the American Type Culture Collection via A. Beyer) was grown on monolayer cultures of HeLa cells prepared in 150-cm^2 tissue culture flasks. Cells were propagated at 37°C in Dulbecco modified minimal essential medium containing 10% tryptose phosphate broth, 10% calf serum, and antibiotics. They were infected in medium lacking serum at a multiplicity of 50 to 200 PFU per cell for 90 min at 37°C. Serum-containing medium was then added, and infection was continued for a total of 48 to 72 h at 37°C. The virus was purified from infected cells by the procedure of Green and Piña (6) except that the genetron step was omitted, CsCl was substituted for RbCl in all density gradient ultracentrifugation steps, and all density gradients were centrifuged at 18°C rather than 4°C. Purified viral prepara-

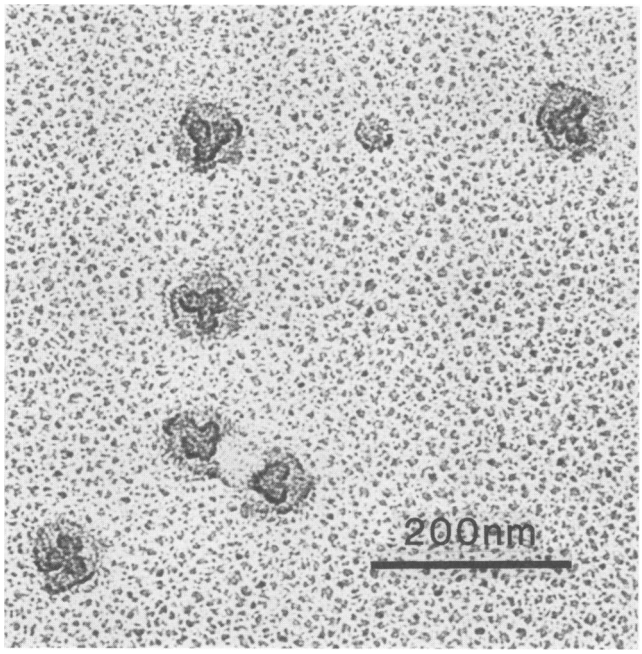

FIG. 3. Representative electron microscope field of etched adenovirus 2 virions prepared for microscopy by rotary shadowing. Intact virions were etched for 20 s with Ar^+, rotary shadowed, and examined in an electron microscope as described in the text.

tions were either used immediately or adjusted to a concentration of 0.5 mg of viral protein per ml and stored at −80°C in 30 mM Tris-hydrochloride (pH 8.1) containing 0.14 M NaCl, 5 mM KCl, 5 mM glucose, 0.7 mM Na_2HPO_4, and 30% glycerol. Preparations yielded 2 to 3 mg of purified virus from five 150-cm^2 HeLa cell monolayers.

Ion etching. Ion etching was carried out with virus particles lyophilized on 400-mesh Formvar and carbon-coated copper electron microscope grids. Purified adenovirus 2 was allowed to adsorb to grids for 1 min at room temperature in 10 mM Tris-hydrochloride buffer (pH 8.1) at a concentration of 0.25 mg of viral protein per ml. Grids were then washed four times in 0.15 M ammonium acetate, frozen in liquid N_2, and lyophilized overnight. Etching was performed in a Technics Hummer V sputter coater operated in the etch mode. Before each use the stage of the instrument was carefully cleaned with steel wool and covered with a single thickness of aluminum foil (Reynolds 625). Electron microscope grids containing adsorbed virus were spaced uniformly on the aluminum foil and etched at a voltage setting of 2 (1,000 V) in an atmosphere of Ar at a pressure of 200 mtorr (ca. 26.6 Pa). This produced a current of 10 mA, which corresponds to an ion flux of 1.95×10^{15} Ar^+ ions per cm^2 per s. The temperature of the stage (room temperature) did not rise appreciably during etching for periods of up to 5 min.

Electron microscopy. All electron microscopy was carried out with shadowed specimens. Pt-Pd (80%:20%) shadows were applied in a JEOL vacuum evaporator at a 30° angle for directional shadowing and a 7° angle for rotary shadowing. Specimens were examined in a JEOL 100cx electron microscope operated at 80 kV. Measurements of all viral and subviral structures were made from positive enlargements of electron microscope negatives, which were taken at a magnification of ×33,000.

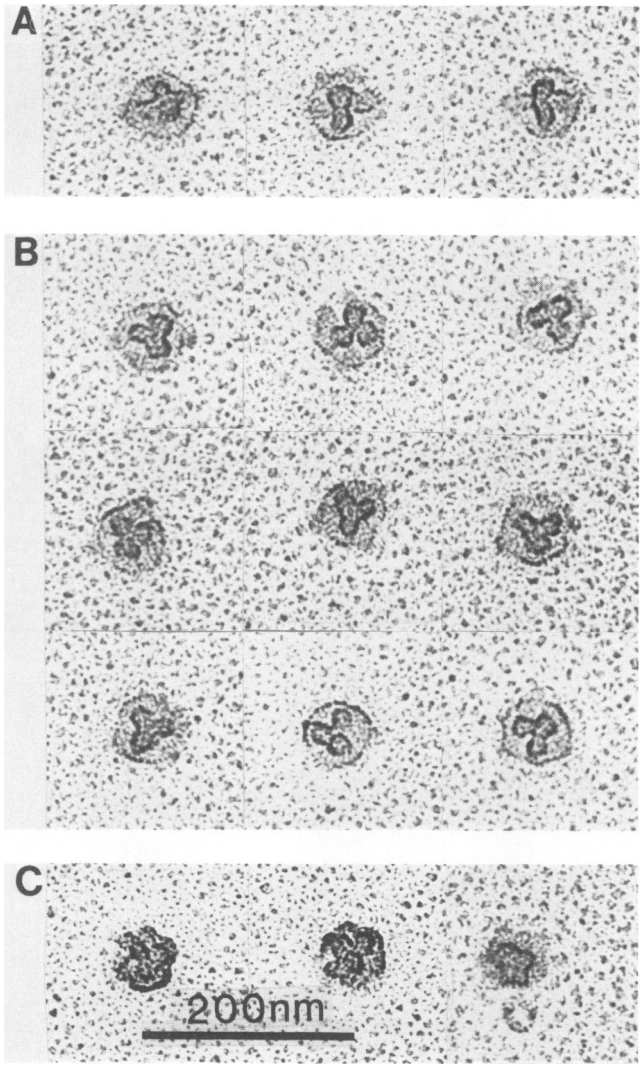

FIG. 4. Adenovirus 2 cores showing large spheres related by (A) two-, (B) three-, and (C) fivefold axes of rotational symmetry. Etching of intact virions (for 20 to 30 s), rotary shadowing, and electron microscopy were carried out as described in the text. The images shown were selected from a sample of 200 etched virions.

RESULTS

Ar^+ etching and directional shadowing. Ion etching, followed by electron microscopy as described above, showed that adenovirus virions were eroded gradually over a period of 2 to 3 min at room temperature. Thereafter, no trace of virus could be detected on electron microscope grids. The apparent virion diameter was found to decrease linearly for the initial 30 to 45 s of etching, as shown in Fig. 1, and more gradually thereafter. Control experiments demonstrated that accelerated ions were required to produce this effect. No change in viral morphology was observed when the ion acceleration step was omitted from the experimental protocol.

Images of etched virions prepared by directional shadowing allowed the process to be divided into distinct stages. Etching (for 10 s) sufficient to reduce the particle diameter by 10 to 20 nm produced a smoothing of virion edges and vertices, as shown in Fig. 2B, but it did not reveal additional

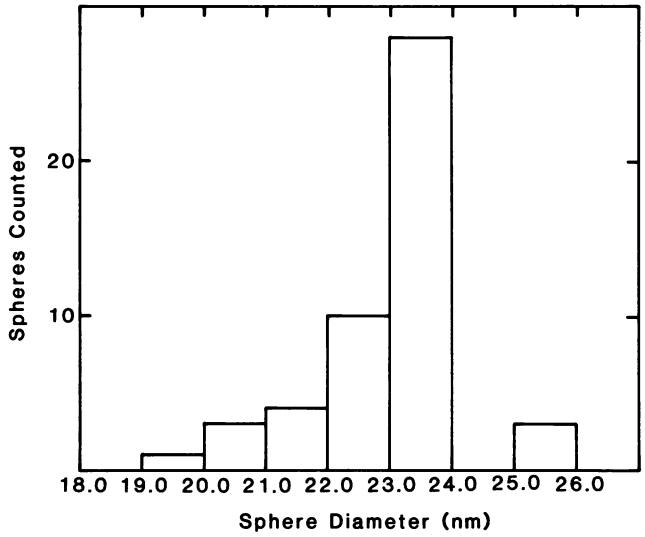

FIG. 5. Large-sphere diameter, as measured in images of etched adenovirus 2. Measurements were made from photographs such as those shown in Fig. 3 and 4. Etching in all cases had been carried out for 20 to 30 s.

substructural features. Etching for 20 s, which reduced the virion diameter by 20 to 30 nm, exposed clusters of uniformly sized spheres which appeared to constitute the virion core (Fig. 2C). Three to six spheres in close proximity to each other were visible in most images, but the size of the overall particle suggested that additional spheres were also present. In many cases sphere clusters were seen to be partially surrounded by a thin covering or shell.

Sphere clusters continued to be visible in images of virions etched for periods (30 to 45 s) sufficient to reduce the particle diameter by 30 to 40 nm (Fig. 2D). The appearance of an outer shell was lost, however, and spheres no longer appeared to be uniform in size. Additional etching resulted in a decrease in sphere size to the point at which the particles themselves were no longer detectable.

Rotary-shadowed preparations. The morphology of the virion core was more clearly resolved in rotary-shadowed preparations than in directionally shadowed ones. Figure 3, for example, shows the result of an experiment in which rotary shadows were applied to virions etched under conditions (20 s of etching) designed to reduce the particle diameter by 15 to 20 nm. Sphere clusters, which were clearly visible in these preparations, were found to be of three types: (i) clusters containing three spheres related by a threefold axis of rotational symmetry (several cores of this type can be seen in Fig. 3), (ii) clusters containing four spheres related by a twofold axis, and (iii) clusters containing six spheres (a central sphere surrounded by five neighbors) related by a fivefold axis. All core images could be readily assigned to one of the three categories. Those with threefold symmetry were most abundant in unselected electron microscope fields. Of 91 cores counted, 70 (77%) demonstrated threefold symmetry, 15 (16%) had twofold symmetry, and 6 (7%) had fivefold symmetry. Figure 4 shows representative examples of the three types of cores observed.

The diameters of individual large spheres were measured in rotary-shadowed preparations such as those shown in Fig. 3 and 4. Measurements of 55 spheres in separate virions yielded an average diameter of 23.0 ± 2.3 nm. The narrow distribution of values observed, as shown in Fig. 5, confirmed the view that the spheres were quite uniform in size. There was no systematic difference in the diameters of spheres measured in cores with two-, three-, or fivefold symmetry.

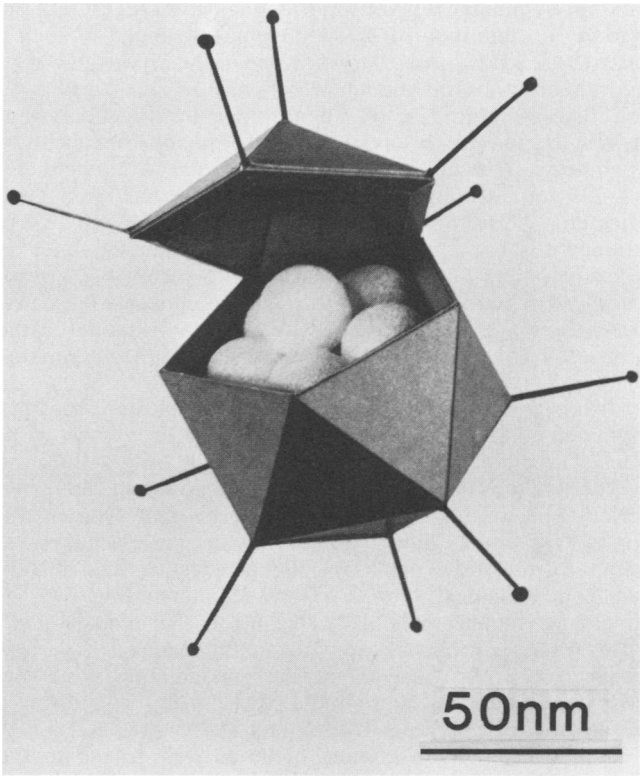

FIG. 6. Model showing the proposed arrangement of large spheres (adenosomes) inside the adenovirus 2 capsid. A portion of the capsid has been raised to expose the core and to illustrate the orientation of adenosomes immediately beneath the capsid vertices.

DISCUSSION

The experimental results described here support the view that large spheres are the basic structural elements of the adenovirus 2 core. Spheres were exposed only after virions were etched deeply enough (10.0 to 15.0 nm) to remove all or most of the viral capsid (12, 14), and spheres with similar morphology were observed in both directionally shadowed and rotary-shadowed preparations. No evidence was obtained for the presence of other structural elements which could constitute the virion core.

Large spheres were found to be uniform in size, with an average diameter of 23.0 nm. They appeared to be fundamentally the same as the large subunits identified by Brown et al. (2) in cores prepared by Sarkosyl extraction of intact adenovirus 2. It is most likely, therefore, that the large spheres contain the viral DNA complexed with virion protein VII and perhaps also with protein V. We suggest that the large spheres be called "adenosomes." This name takes into account the generic similarity between the spheres and cellular nucleosomes (10), but it emphasizes the fact that they are quite different structures, which are unique, as far as is known, to adenoviruses.

Ion etching experiments showed that the large spheres (adenosomes) were packed tightly together in virion cores, where they were related by two-, three-, and fivefold axes of rotational symmetry. The sizes of individual adenosomes

and the symmetry relationships among them suggest that the virion core consists of 12 adenosomes arranged in such a way that each is directed toward one of the 12 vertices of an icosahedron. In this model, which was originally proposed by Brown et al. (2), all adenosomes occupy equivalent positions, and they are related by the three observed axes of symmetry. When constructed from spheres with a diameter of 23.0 nm, the suggested core structure has an outside diameter (vertex-to-vertex distance) of 67.0 nm and a face-to-face distance of 58.2 nm. It can be accommodated inside the adenovirus 2 capsid, whose vertex-to-vertex distance we measure to be 86.5 nm (Fig. 1), if each adenosome is located just below a capsid vertex. Figure 6 shows a model of the proposed arrangement of adenosomes inside the adenovirus 2 capsid.

It is significant that adenosomes demonstrated threefold symmetry in a high proportion (77%) of etched virus particles. Threefold symmetry is expected to be observed for the vertices of icosahedral virions lying face down, the preferred orientation, on electron microscope grids (20). Demonstration of threefold symmetry in most core images, therefore, supports the view that core adenosomes lie immediately adjacent to capsid vertices. Cores demonstrating two- or fivefold symmetry would, according to this model, result from virus particles resting on an edge or on a vertex, respectively. Virions with the latter orientation should be less stably mounted and should, therefore, occur at lower frequency than particles resting on a face.

Application of ion etching methods was crucial to the development of the studies described here. Use of Ar^+ ions allowed the adenovirus 2 capsid to be gradually eroded in a way that permitted core structures to be visualized in their native state. Virions did not need to be chemically disassembled before core structures were visualized. Etching could be carried out with lyophilized or aldehyde-fixed virus particles, and considerable flexibility existed in the rate at which virions could be etched. The etching rate depended critically on the total current, which could be varied over a wide range (at least 1 to 25 mA). Virus erosion by Ar^+ was nonspecific in that it did not depend on the particular biochemical properties of virion surface components. It is quite possible, therefore, that in the future, ion beams may be usefully employed to examine the structures of other viruses and of other biological materials as well.

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