5. Griess, G. A., P Serwer, and P. M. Horowitz. 1985. Binding of ethidium to bacteriophage T7 and T7 deletion mutants. *Biopolymers*. In Press.
6. Scatchard, G. 1949. The attraction of proteins for small molecules and ions. *Ann. NY Acad. Sci.* 51:660–672.
7. McCall, P. J., and V. A. Bloomfield. 1976. Kinetics of proflavin binding to bacteriophages T2L and T4D. *Biopolymers*. 15:2323–2336.
8. Moore, J. W., and R. G. Pearson. 1981. Kinetics and Mechanism. John Wiley & Sons, Inc., New York.

STRUCTURAL STUDIES OF ADENOVIRUS TYPE 2 AND AN ASSEMBLY MUTANT

C. BERTHET-COLOMINAS,* P. BOULANGER,‡ C. DEVAUX,*‡ B. JACROT,* AND P. A. TIMMINS§
**European Molecular Biology Laboratory Grenoble Outstation, c/o Institut Laue-Langevin, 38042 Grenoble Cedex, France; ‡Laboratoire de Virologie, Institut National de la Santé et de la Récherche Medicale, 59045 Lille, France; and §Institut Laue-Langevin, 38042 Grenoble Cedex, France*

Adenovirus type 2 is an icosahedral mammalian virus containing 22.2 × 10^6 d of genomic DNA and some 10 structural proteins having a total molecular mass ~140 × 10^6. The structure of the major coat protein, the hexon, representing some 65% of the total protein, has been solved to 2.9 Å resolution by Burnett and colleagues (1). High-resolution studies are also being carried out on the fiber protein that attaches to the fivefold apices of the virus (2). Here we describe experiments using x-ray and neutron diffraction, electron microscopy with image reconstruction, and biochemical techniques aimed at investigating various aspects of the organization of adenovirus 2. In addition to native virus we have investigated a temperature-sensitive mutant H_2ts112 which, when grown at the nonpermissive temperature, contains only a small piece of DNA and lacks at least one major protein. We have also studied groups of nine hexons (GON) which are whole facets of the viral icosahedron obtained by treating the particle with sodium deoxycholate (3).

MATERIALS AND METHODS

Viral particles were grown in KB cells at 37°C for 40 h (human adenovirus type 2 wild type) or at the nonpermissive temperature, 38.5°C, for 20 h (mutant H_2ts112). Purification and isolation of viral particles and GON was achieved as described previously (4). Neutron scattering measurements were carried out at the D11 small-angle scattering instrument of the Institut Laue-Langevin. Sample-detector distances of 2–20 m and incident neutron wavelengths of 5 and 10 Å were used. Sample concentrations were 0.2–2.0 mg/ml. X-ray scattering experiments were performed using a double focusing system on an Elliot GX13 rotating anode tube. Specimen-to-film distance was 100 mm. Electron microscopy in amorphous ice (5) was carried out using the Phillips EM400 at European Molecular Biology Laboratory, Heidelberg.

RESULTS

Small-angle neutron scattering data were measured from solutions of native Ad2 and the mutant dialyzed against the same buffer made up in 0%, 25%, 70% and 100% D_2O/H_2O mixtures. These scattering curves were fitted by spherical shell models (Fig. 1) as previously described for the native structure (4). The intensity at zero scattering angle yields a molecular weight of 157 × 10^6 for the native particle and 125 × 10^6 for the mutant. Using these values, the molecular weights of protein and DNA can be derived for the individual shells as shown in Table I. The radial organization of native and mutant viruses shows the latter to be a much more hollow particle encapsidating only about one fifth of the normal mass of DNA. The protein distribution is somewhat surprising: the mutant contains some 16 × 10^6 of protein in the core although it lacks the major core protein VII. This can be explained only by assuming that the immature precursor protein pVI is in the core and has not been inserted in the capsid. Similarly, the

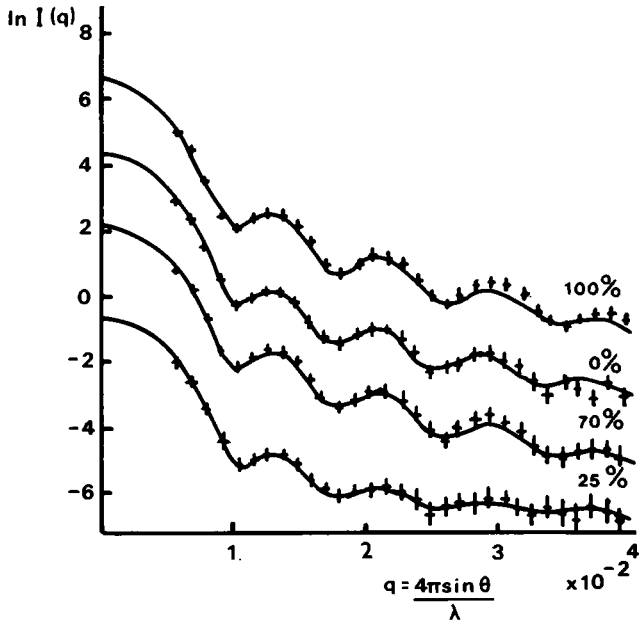

FIGURE 1 Small-angle neutron scattering curves from solutions of the adenovirus type 2 mutant H_2ts112 in various H_2O/D_2O mixtures. The dotted lines represent the experimental data and solid lines the fits of the model described in the table.

TABLE I
RADIAL ORGANISATION OF PROTEIN AND DNA IN NATIVE ADENOVIRUS AND H_2ts112

Shell radii	Native virus		H_2ts112	
0–303	DNA	22.2×10^6	DNA	4.4×10^6
	Protein	22.3×10^6	Protein	16×10^6
303–348	Protein	30.9×10^6	Protein	12×10^6
348–420	Protein	83.7×10^6	Protein	92×10^6

high density in the outer protein shell of the mutant implies a movement of protein on maturation to the native virus. X-ray small-angle scattering from the the mutant particle showed that the small piece of encapsidated DNA, unlike that of the native particle DNA, does not give rise to the 1/29 $Å^{-1}$ maximum (4) and is therefore not ordered in a similar manner. Considering the extremely icosahedral shape of the virus as seen in the electron microscopy, we can convert the spherical shell model into an equivalent scattering icosahedron (4) which gives us a measure of the overall real particle dimensions. For both the mutant and native virus this yields a distance between fivefold vertices of 520 Å. This value is confirmed by electron-microscopy measurements from specimens in amorphous ice that give 515 ± 15 Å, corresponding to a water content of ~0.3 g/g of virus, which is reasonable. The distance between fivefold axes also yields a hexon-hexon distance ~103 Å which agrees well with values obtained from isolated GON either by x-rays (98–100 Å) or by EM in amorphous ice (101 Å). They are larger than expected from a close packing of the hexons, which would correspond to ~89 Å. This implies that another protein, perhaps protein IX, plays a role in the capsid integrity. Indeed, in image reconstructions of GON we see rather tenuous contacts between adjacent hexons. Colby and Shenk (6) observed, however, that the mutant H_5dl313, which lacks protein IX, is able to form rather fragile but complete capsids. We do not know the dimensions of these capsids and must conclude that this observation merely shows that close packing is possible, and that a looser packing, perhaps modulated by protein IX, is found in the native virus.

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REFERENCES

1. Burnett, R. M. 1984. Structural investigations on hexon, the major coat protein of adenovirus. *In* Biochemical Macromolecules and Assemblies. A. McPherson and F. A. Jurnak, editors. Vol. I. John Wiley & Sons, Inc., New York, NY.
2. Devaux C., C. Berthet-Colominas, P. A. Timmins, P. Boulanger, and B. Jacrot. 1984. Crystal packing and stoichiometry of the fibre protein of adenovirus type 2. *J. Mol. Biol.* 174:3.
3. Boulanger, P. A., C. Devaux, and M. H. Loucheux-Lefebvre. 1978. Comparative optical properties of free and assembled hexon capsomers of human adenovirus type 2. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 85:52–56.
4. Devaux, C., P. A. Timmins, and C. Berthet-Colominas. 1983. Structural studies of adenovirus type 2 by neutron and x-ray scattering. *J. Mol. Biol.* 167:119–132.
5. Adrian, M., J. Dubochet, J. Lepault, and A. W. McDowall. 1984. Cryoelectron microscopy of viruses. *Nature (Lond.)*. 308:32–36.
6. Colby, W. W., and T. Shenk. 1981. Adenovirus type 5 virions can be assembled in vivo in the absence of detectable polypeptide IX. *J. Virol.* 39:977–980.

PROGRESS IN UNDERSTANDING ADENOVIRUS ARCHITECTURE

ROGER M. BURNETT, JAN VAN OOSTRUM, AND MICHAEL M. ROBERTS

Department of Biochemistry and Molecular Biophysics, College of Physicians and Surgeons, Columbia University, New York, New York 10032

The icosahedral adenovirus virion (Fig. 1) is formed from at least 10 different polypeptides and has a total molecular mass of 149,000 d.[1] The detailed architecture is being explored by x-ray crystallography, electron microscopy, and biochemistry (1). A 2.9 Å electron density map of the major coat protein, hexon, has been obtained for adenovirus type 2. The molecular envelope (2) reveals that, although trimeric, hexon has a basal region exhibiting pseudo-hexagonal symmetry. An upper triangular region, directed outward when the molecule is incorporated into the capsid, provides a recognizable feature for the determination of hexon organization using the electron microscope. The three-dimensional structure determination was recently completed,[2] and the chain tracing reveals 859 of the 967 amino acids. The base is formed from three pairs of nonidentical domains, whereas three identical domains shape the top. The majority of the missing amino acids, and the regions of heterologous sequence, lie within the hexon tops and thus the outer capsid surface.

A model for the overall arrangement of hexons in adenovirus has been derived (3), and confirmed using the

[1]van Oostrum, J., and R. M. Burnett. 1985. Molecular composition of the adenovirus type 2 virion. Manuscript submitted for publication.

[2]Roberts, M. M., J. L. White, M. G. Grutter, and R. M. Burnett. 1985. Three-dimensional structure of adenovirus hexon. Manuscript in preparation.

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