JOURNAL OF VIROLOGY, Oct. 1970, p. 569-571

Vol. 6, No. 4
Printed in U.S.A.

Molecular Weight of Adenovirus Type 2 Hexon Polypeptide

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Received for publication 28 May 1970

The fully dissociated hexon polypeptide of adenovirus type 2 has a molecular weight of 129,000 (±10,000) daltons as determined by high-speed sedimentation equilibrium in 6 M guanidine.

Hexon, the major capsomere of adenovirus type 2, appears to be composed of multiple identical polypeptide chains (7). The molecular weight of the individual hexon polypeptide dissociated in sodium dodecyl sulfate (SDS) is 120,000 daltons, based on its mobility in SDS-containing acrylamide gels (7, 10). Dissociation of hexon in 6 M guanidinium hydrochloride and subsequent electrophoresis on SDS-acrylamide gels yielded similar results (4). Other data consistent with a large hexon polypeptide were obtained in studies on the number of tryptic peptides and the synthesis time of the hexon polypeptide (4). On the other hand, a molecular weight of approximately 25,000 daltons has been suggested based on the size of the polyribosomes synthesizing viral proteins and on sedimentation values for nascent viral peptides (11).

In view of the conflicting reports, the molecular weight of the hexon polypeptide has been determined under strong dissociating conditions in 6 M guanidinium hydrochloride by high-speed sedimentation equilibrium (12). Sedimentation equilibrium permits the calculation of molecular weight without the need to measure sedimentation values and diffusion coefficients. Both of these latter hydrodynamic properties are very sensitive to the precise shape of molecules, and if they are measured under different conditions or calculated from hypothetical ideal models, as is often done, there may be significant inaccuracies. There was the additional advantage in sedimentation equilibrium that microgram quantities of dissociated hexon, in 6 M guanidinium hydrochloride and deuterated water, could be used to determine the partial specific volume and molecular weight simultaneously (3).

[1] Recipient of Health Research Council Award from the City of New York.

[2] Recipient of Faculty Research Award from the American Cancer Society.

[3] Recipient of Public Health Service Career Development Award.

Hexon capsomeres were purified from adenovirus type 2-infected HeLa cells by chromatography twice on diethylaminoethyl-cellulose (7). Purity was examined by electrophoresis in two acrylamide gel systems: (i) neutral SDS (7) which separates dissociated proteins by size only (10) and (ii) tris(hydroxymethyl)aminomethyl chloride (Tris) at *p*H 8.9 (2), which separates native proteins by size and electrical charge. Gels were heavily loaded with sample in an attempt to detect minor components. With smaller amounts of sample, the major band appears sharper and shows no indication of heterogeneity. The SDS gel revealed what may be a minor contaminant on the leading edge of the hexon band (Fig. 1). Hexon was dissociated by dialysis against 5 to 6 M guanidine solutions containing either 0.15 M mercaptoethanol, 0.3 M iodoacetamide (recrystallized from ethanol), or dithiothreitol (8) at *p*H readings from 7.9 to 10.1 (see Table 1 for details). The final concentration of protein was 0.24 to 0.85 mg/ml.

Since the partial specific volume ($\bar{v}$) is necessary to calculate the molecular weight, $\bar{v}$ was determined directly in 6 M guanidine. Because of the small amount of purified protein available, a modification of the heavy water method of Edelstein and Schachman (3) was used in which the solute distribution at equilibrium in both deuterium oxide and water can be used to calculate $\bar{v}$ in addition to the molecular weight. Hexon was dialyzed against 6 M guanidinium hydrochloride, 0.15 M mercaptoethanol, and 0.01 M Tris chloride buffer (*p*H 8.3) dissolved in deuterium oxide to raise the density of the solvent. Calculation of the results requires a knowledge of the ratio of the molecular weight of hexon in deuterium oxide to the molecular weight in water. This ratio is a function of the number of exchangeable hydrogens in the protein and was calculated as suggested (3). However, the per

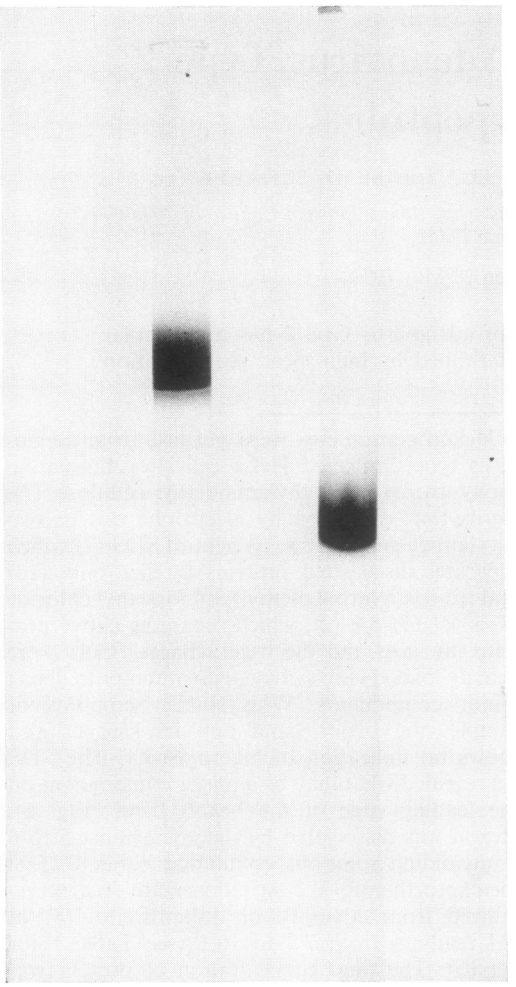

FIG. 1. *Acrylamide gel electrophoresis of hexon. Hexon, purified twice by DEAE-cellulose chromatography, was electrophoresed on either a 5% SDS containing acrylamide gel (left) or on a 5% Tris chloride-containing acrylamide gel (right) to test for purity of the preparation. The origin is at the top and migration is toward the anode at the bottom (Maizel, 1969). The gels were stained with Coomassie blue.*

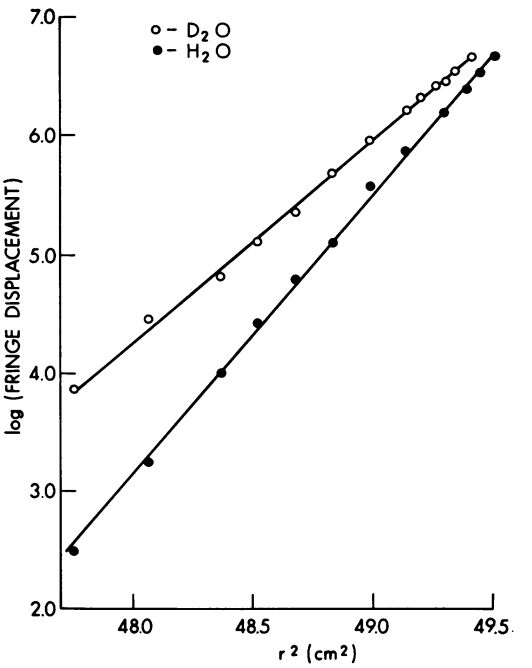

FIG. 2. *Molecular weight determination of hexon subunit in either D_2O or water containing solvents. Hexon subunit dissociated in 6 M guanidinium hydrochloride, 0.15 M mercaptoethanol, 0.01 M Tris buffer (pH 8.1 to 8.3), and in either water or D_2O was centrifuged at 23,150 rev/min for determination of its molecular weight and partial specific molar volume. The ordinate is the natural logarithm of the fringe displacement plotted against the square of the distance from the center of rotation (abscissa).*

cent of deuterium of all isotopic hydrogen in a solution of 6 M guanidine in deuterium oxide is not 9.99 as for the deuterium oxide alone, but is 63.4, assuming that all of the hydrogens of guanidine are readily exchangeable with the solvent. This assumption was substantiated by examining, in a Varian A 60 (60 MHz) spectrophotometer, the nuclear magnetic reasonance (NMR) spectrum of 6 M guanidine in deuterium oxide at ambient temperature. The amount of hydrogen-deuterium exchange was measured by quantitating the number of hydrogens in the product (DHO and HHO) in comparison to the hydrogens remaining in guanidine. The theoretical ratio (moles of H in DHO and HHO/moles of H in guanidine) for complete hydrogen-deuterium exchange was 1.94 and that obtained by NMR measurement was 1.95. $\bar{v}$ was also calculated by using the amino acid composition of the protein (9; Horwitz, *unpublished data*) and partial specific volumes of the amino acids (1). The densities of the solutions were determined by direct weights of samples in volumetric glassware standardized against water.

Equilibrium studies were performed in a Beckman model E analytical ultracentrifuge by using the Rayleigh interference optical system to measure the concentration distribution of the solute. All samples were centrifuged at either 23,150 or 24,630 rev/min at 25 C, and reached equilibrium within 48 hr. Interference patterns were photographed on Kodak spectroscopic plates, type 11-G, and fringe displacement was measured on a Nikon Shadow Microcomparator.

The molecular weight of the hexon polypeptide

TABLE 1. *Molecular weight of hexon polypeptide subunit in 5 to 6 M guanidine*

Molecular weight					
Mercaptoethanol (*p*H 8.1)[a]		Iodoacetamide (*p*H 7.9)[b]		Dithiothreitol (*p*H 10.1)[c]	
0.721[d]	0.737[d]	0.721	0.737	0.721	0.737
108,000 111,000 123,000	122,000[e] 125,000[h] 139,000[i]	125,000	141,000[f]	105,000	119,000[g]

[a] Composition: 6 M guanidine, 0.15 M mercaptoethanol, 0.01 M Tris, *p*H 8.1.
[b] Composition: 6 M guanidine, 0.3 M iodoacetamide, 0.02 M sodium phosphate, *p*H 7.9.
[c] Composition: 5 M guanidine, 0.05 M dithiothreitol, 0.02 M potassium carbonate, 0.01 M potassium chloride, 0.001 M EDTA, *p*H 10.1.
[d] $\bar{V}$ of 0.721 was calculated from the amino acid composition. The $\bar{v}$ of 0.737 was determined by using the heavy water method (3) at three protein concentrations (0.27 to 0.55 mg/ml).
[e] Amount: 0.73 mg/ml. [f] Amount: 0.45 mg/ml. [g] Amount: 0.85 mg/ml.
[h] Amount: 0.37 mg/ml. [i] Amount: 0.24 mg/ml.

in 5 to 6 M guanidine, under a variety of conditions and protein concentrations, was 114,000 (± 9,000) daltons by using the $\bar{v}$ of 0.721 calculated from the amino acid composition (Table 1). The average molecular weight was 129,000 (± 10,000) by using the $\bar{v}$ of 0.737 (± 0.035) measured by the heavy water technique (Table 1, Fig. 2).

The presence of 0.15 M mercaptoethanol in 6 M guanidine at *p*H 8.1 is sufficient to reduce most multichain proteins to monomeric units (5). In studies of the subunits of tryptophanase, however, the polypeptides were further reduced in size by increasing the *p*H to 10.5 (8). The results of equilibrium centrifugation at *p*H 8.1 and 10.1 were in substantial agreement, suggesting that there were no resistant interchain disulfide bonds at *p*H 8.1. Since the molecular weight of native hexon alkylated with iodoacetamide and dissociated in 6 M guanidine was similar to the values after reduction with mercaptoethanol, there are probably no interchain disulfides in the native molecule.

Thus, the results of equilibrium centrifugation under a variety of conditions are consistent with the molecular weight determinations obtained by SDS-acrylamide gel electrophoresis. We have also determined the molecular weight of undissociated hexon by sedimentation equilibrium in the absence of guanidine and obtained an estimate of 374,000 (Horwitz et al., *unpublished data*), which falls between other estimated values [310,000 (6); 400,000 (9)] and is nicely consistent with the suggestion that the morphological subunit called hexon is composed of three identical polypeptide chains (7).

We thank John Pesando for help with the nuclear magnetic resonance determinations.

This work was supported by Public Health Service grants AI4153 and AI5231 from the National Institute of Allergy and Infectious Diseases and CA11512 from the National Cancer Institute, by the American Cancer Society, and by the National Science Foundation.

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