

Studies on Equine Adenovirus

II. Isolation of Hexon Antigen

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Owing to the group-specific antigen of Mastadenovirus, hexon cross-reacts to a great extent in the complement fixation (CF) test. An equine adenovirus isolated by the present authors from a Thoroughbred colt with pneumonia reacted reciprocally with a canine adenovirus in the CF test [3], but the CF antigen of the equine adenovirus has not been isolated as yet. This paper presents the physical properties of the CF antigen and the morphology of the hexon examined by electron microscopy.

The adenovirus isolated was grown in the equine fetal dermis cell, as previously described [3]. It was purified from the infected cell extracts by isopycnic banding of virus particles in cesium chloride (CsCl) density gradient. The virus particles were successfully disrupted by dialysis against distilled water at 4°C for 72 hr. The suspension of the disrupted virus was further layered on a 30 to 60% sucrose gradient. Centrifugation proceeded at 25,000 rpm for 3 hr in a Hitachi 65P preparative ultracentrifuge. Twenty fractions were collected from the gradient and tested for the hemagglutination (HA) and CF activities after removal of the sucrose by dialysis against distilled water. The CF activity of each fraction was determined by the method of Norrby [7]. The cross CF test and preparation of antiserum against the equine and canine adenoviruses were carried out in the same manner as described before [3]. The HA activity was determined with guinea pig erythrocytes at a concentration of 0.5% in 0.01 M Dulbecco's phosphate-buffered saline. The CF activity appeared as a single band in the lower density of sucrose gradient. It was almost completely separated from HA activity (Fig. 1). No infectivity could be detected in any fraction with CF activity.

The fractions with CF activity were pooled and used as antigen for the cross CF test. The separated CF antigen reacted at 1:128 with the antiserum against the canine adenovirus. This serological relationship was consistent with that clarified by antigens of these viruses in the previous report [3].

The fraction with CF activity from the sucrose gradient was further layered on a CsCl density gradient, and centrifuged at 50,000 rpm for 18 hr. Twenty fractions
were collected and examined for density, CF activity and absorbancy at a wavelength of 280 nm with a Hitachi 124 spectrophotometer. The CF antigen had a buoyant density of 1.34 g/cm³ in CsCl, corresponding to a maximum extinction at 280 nm (Fig. 2).

The CF antigen was also negatively stained with 1% phosphotungstic acid at pH 7.0 and examined in a Hitachi HU-12 electron microscope. The grid was placed in the holder so that the specimen-filmed side might face the screen. Photographs were taken in the conventional manner. Electron microscopically, the CF antigen consisted of groups of nine hexons each with threefold rotational symmetry (Fig. 3). No peripentonal hexons, however, were observed in this condition.

The groups of nine hexons, also named "nineomers" or "nonamers", have been released only from the human adenoviruses treated with acetone, urea, pyridine, sodium lauryl sulfate, or by heating at 56°C [4, 5, 9–11]. They were presumably from a single face of the 20 triangular facets making the adenovirus icosahedron.

This paper first shows the morphology of the groups of nine hexons from adenovirus other than that of human origin. Although these groups must have an equal probability of presenting a left- or right-handed configuration on the grid, most of them were in left-handedness in the present observation. This suggests that the group of nine hexons may be a polar oligomer in the inside surface of which is hydrophobic and which is attached to the grid with preference. Franklin et al. [2] and Pereira and Wrigley [8] also showed the left-handedness of all the groups of nine hexons, except a few which were in right-handedness. Fig. 4 presents an exceptional right-handed group of nine hexons.

Threefold symmetry of the group of nine hexons reported by Crowther and Franklin [1] was also clearly seen in the present observation. Valentine and Pereira [12] mentioned that the hexon of human adenovirus was about 8.0 nm in diameter and the center-to-center spacing between the hexons about 8.2 nm. These values were about 7.0 nm and 7.5 nm, respectively, in the present observation. Nermurt [6] proposed a model of hexon which had an axial hole of 2.5 to 3.5 nm in diameter at the top and of 1.0 to 1.5 nm at the bottom. In the present observation the axial hole was 1.5 nm in diameter, but its role remained unknown.
References


Explanation of Figures

Fig. 3. Groups of nine hexons in left-handedness separated from the equine adenovirus. Phosphotungstic acid staining, ×1,000,000. Bar represents 10 nm.

Fig. 4. Exceptional group of nine hexons in right-handedness (●). Phosphotungstic acid staining, ×230,000.