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Brief Note

Light Particles in a Regular Population of Adenovirus Type 2

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ABSTRACT. Purified preparation of human adenovirus type 2 were resolved in light and regular particles on equilibrium density gradient centrifugation in CsCl. The difference in their densities is less than 0.01 g/cm^3, suggesting that there is a slight difference in ratio of protein to deoxyribonucleic acid and/or conformation of the virus.

Structure of adenovirion has been studied in detail by a number of investigators. In the course of purification of the adenovirus, we found that the ordinary purified preparation of adenovirion contained slightly light particles in the regular virion population with respect to their buoyant densities.

Human adenovirus type 2 was propagated in KB cells grown in Joklik modified medium for suspension supplemented with 7% new born calf serum for

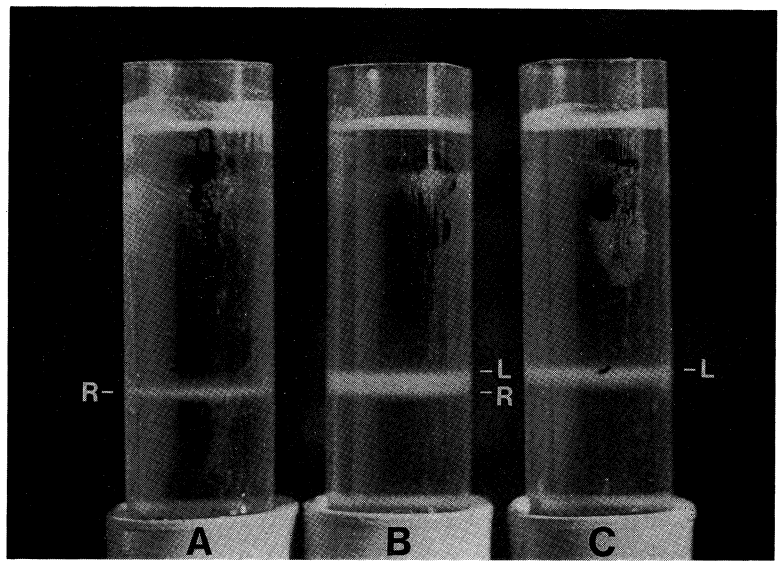

Figure 1. Regular and light particles separated in a density gradient of cesium chloride. Purified regular and/or light particles were mixed with cesium chloride in 0.03 M Tris-HCl (pH 7.5), and centrifuged at 40,000 rev/min for 24hr at 4° in a Beckman SW 50.1 rotor. A : regular (R) particles only, B : mixture of regular (R) and light (L) particles, C : light (L) particles only.

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48 hr and purified by two cycles of equilibrium density gradient centrifugation in CsCl[2]. After the first CsCl centrifugation, main virus band was isolated and further purified by the second CsCl centrifugation in a Beckman SW41 rotor. The upper part of the rebanded virus was isolated as the light particles and the

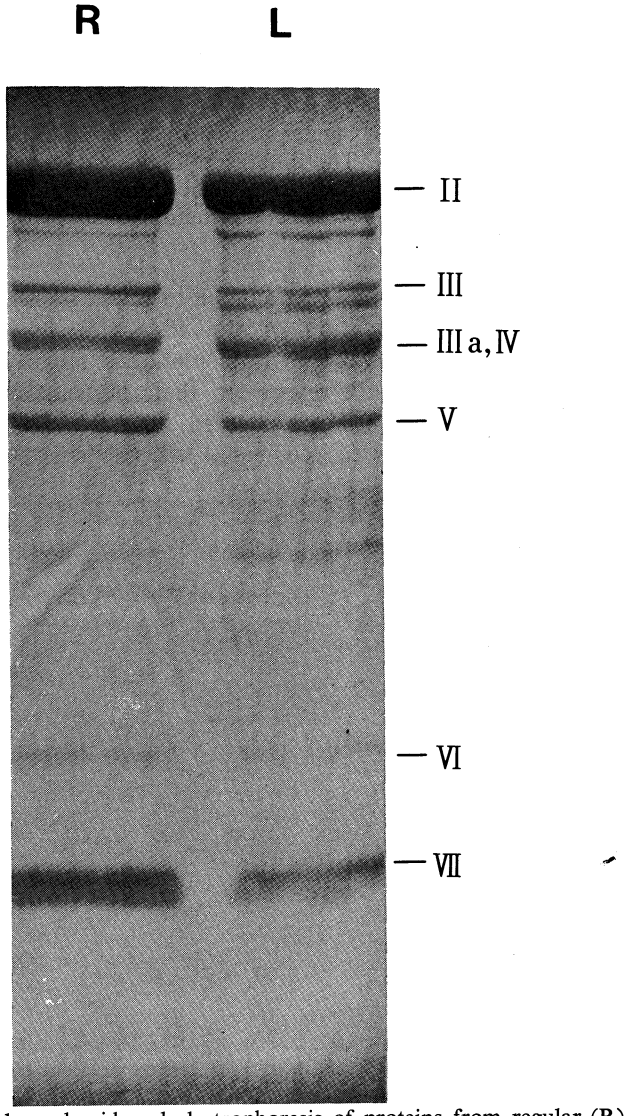

Figure 2. SDS-Polyacrylamide gel electrophoresis of proteins from regular (R) and light (L) particles. Isolated particles were boiled with 2% SDS, 5% 2-mercaptoethanol, and 10% glycerol in 60 mM Tris-HCl, pH 6.8, and 10% polyacrylamide gels were cast and run as described[1]. After electrophoresis, polyacrylamide gels were stained with Coomassie brilliant blue.

lower part as the regular particles.

Regular and light particles were mixed in CsCl solution and spun in a SW50.1 rotor. Figure 1 shows that a mixture of the regular (R) and light (L) particles formed two separate bands in a CsCl density gradient as shown in (B).

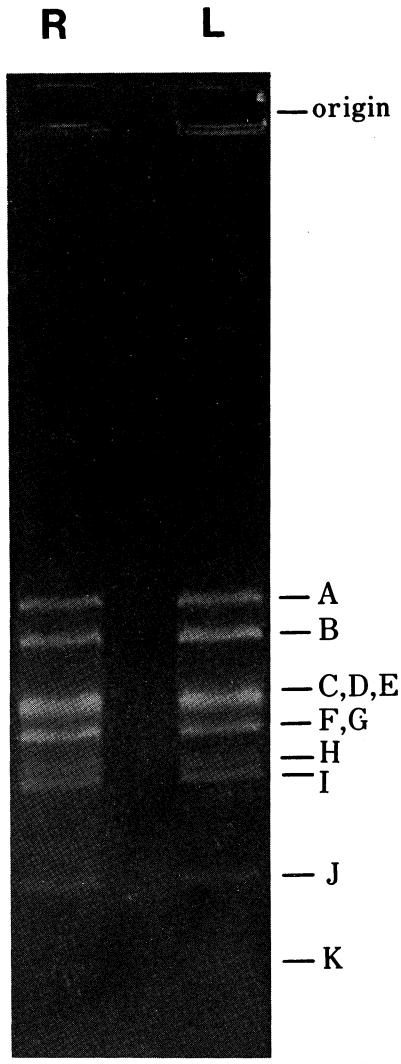

Figure 3. Comparison of *Hind* III fragmentation profiles of DNA from regular (R) and light (L) particles. Purified DNA was digested with restriction endonuclease *Hind* III and fragments were separated by electrophoresis in 1.0% agarose gels in TBE buffer (50 mM Tris, 50 mM boric acid, 1 mM EDTA, pH 8.3). The gels were stained with ethidium bromide and photographed by ultraviolet light. R : regular particle DNA digested with restriction endonuclease *Hind* III, L : light particle DNA digested with restriction endonuclease *Hind* III.

The result indicates that even if purified preparation of virus banded, though slightly dispersed, in a fairly concentrated disc, it is capable of giving rise to two close but distinct bands in a CsCl density gradient upon recentrifugation in a small quantity for better resolution. Figure 1 (C) also shows that the band of light particles is more dispersed than that of regular particles and this may imply that light band cosists of more heterogenious population of particles. Buoyant density of regular and light particles was 1.3397 and 1.3310 (g/cm^3) respectively, and the difference was less than 0.01 (g/cm^3). The small difference suggests that there may be a small difference in their protein to DNA ratio or in conformation.

To know the protein constituents, protein from these particles were analyzed by SDS-polyacrylamide gel electrophoresis for comparison as shown in Figure 2. The results show that there are some differences in the pattern of banding. An increase in a band identical or being overlapped with polypeptides IIIa and IV, another migrated between polypeptides II and III, and the third between polypeptides III and IIIa-IV block were observed for the light particle. As protein of regular particles gives rise to some faint bands in these same locations, the proteins of light particle might be contamination of the host KB cell protein, or these unidentified bands might be partially degraded products of protein II and III.

As difference in buoyant densities between Ad-2 and Ad-12 was very small (0.006 g/cm^3) as reported by Doerfler[3], the possibility cannot be excluded that light particles might be contaminated virions of Ad-12. To find whether these particles are of identical virus serotype or not, phenol isolated DNA was digested with restriction endonuclease *Hind* III, and analyzed by agarose gel electrophoresis as shown in Figure 3. Gel profiles of DNA fragments from regular and light particles are indistinguishable, indicating that these DNA have the same base sequence. The results imply that both regular and light particles are derived from the same virus origin.

To compare the structure, we took the electron micrograph of these two particles, however we could not detect any differences in the two kinds of virions (data not shown).

The data presented in this report show that a purified preparation of human adenovirus type 2 contains slightly light particles in a regular virion population. The light particles are clearly different from top components, that is, empty capsids and incomplete virions because of their buoyant densities as reported previously[4,5], but the light particles might correspond to the young virion described by Ishibashi and Maizel[6] though further investigation is necessary. Small difference in buoyant densities of regular and light particles can be ascribed to a small difference in their protein to DNA ratio and/or in conformation of the virions.

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