Echovirus 11 Dense Particles: Isolation and Preliminary Characterization

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SUMMARY

Dense particles (density 1.44 g/ml) were isolated during purification in CsCl of echovirus 11 produced in HeLa cells. The dense particles had similar antigenic properties and similar RNA and protein composition to standard (density 1.33 g/ml) echovirus 11 particles. They differed from standard particles in their higher buoyant density, lower infectivity and slightly smaller diameter. In contrast to other picornavirus dense particles, echovirus 11 dense particles were present as a major component of virus population. This high ratio of dense particles was found only in virus from HeLa cells which produced non-haemagglutinating echovirus 11. Haemagglutinating echovirus 11 from primary monkey kidney (MK) cells did not contain any detectable levels of dense particles.

Dense particles, i.e. particles of higher buoyant density (density 1.44 g/ml) in CsCl than standard particles (density 1.33 g/ml), have been described for several picornaviruses: poliovirus, coxsackie B5 virus, mengovirus, bovine enterovirus, and swine vesicular disease virus (SVDV) (Rowlands et al., 1975; Yamaguchi-Koll et al., 1975; Wiegers et al., 1977; Perez-Bercoff et al., 1978). Antigenic properties and chemical properties, such as amount of RNA, polypeptide composition, and RNA to protein ratio, are similar for dense and standard particles. It was suggested (Rowlands et al., 1975; Perez-Bercoff et al., 1978) that higher buoyant density results from conformational differences between dense and standard particles, dense particles having a more open capsid structure leading to a greater interaction of virus RNA with Cs+ ions (Rowlands et al., 1975; Yamaguchi-Koll et al., 1975; Wiegers et al., 1977, 1978; Mapoles et al., 1978).

In this communication, we describe the isolation from CsCl density gradients of a dense (density 1.44 g/ml) component of echovirus 11 produced in HeLa cells. To determine whether these particles were similar to dense particles described for other picornaviruses, various properties of these particles were compared with those of standard echovirus 11 particles.

Echovirus 11 (prototype strain Gregory) was passaged in monolayers of HeLa cells at an m.o.i. of 1 to 5. Virus obtained after 10 consecutive passages in HeLa cells (HeLa 10) was purified as described previously (Cova & Aymard, 1980). Briefly, 48 h after infection extracellular virus was concentrated by ultrafiltration (Amicon DC2 system) after removal of cell debris by low-speed centrifugation (30 min at 2500 g) and then by ultracentrifugation for 3-5 hours at 170000 g in an L 3.40 Beckman ultracentrifuge. Concentrated virus was resuspended in PBS (isotonic phosphate-buffered saline: 0.12 M-NaCl, 0.02 M-phosphate pH 7.2), sonicated for 1 min (Branson Sonicator 50 W) and then layered on to a preformed 10 ml linear gradient of CsCl (PBS pH 7.2). Gradients were centrifuged to equilibrium for 18 hours at 150000 g. About 20 fractions were collected and tested for absorbance at 260 nm and 280 nm, refractive index, infectivity and haemagglutinating (HA) activity. Fractions containing virus were pooled, dialysed overnight against PBS and then examined by electron microscopy.

The sedimentation behaviour of echovirus 11 (HeLa 10) in a CsCl gradient is illustrated in Fig. 1. The top component, at a density of 1.12 to 1.20 g/ml, contained mainly cell
Fig. 1. Sedimentation of echovirus 11 (HeLa 10) in CsCl gradient. Echovirus 11 suspended in 2 ml PBS pH 7.2 was layered on to a preformed 10 ml linear gradient of CsCl (PBS pH 7.2), and centrifuged to equilibrium for 18 h at 150000 g in the SW41 rotor of L 3.40 Beckman ultracentrifuge. About 20 fractions were collected and tested for absorbance at 260 nm and 280 nm, haemagglutinating activity and infectivity. The density of each fraction was estimated from its refractive index. Fractions containing virus were pooled, dialysed overnight against PBS pH 7.2 and concentrated by ultracentrifugation. The pellet was resuspended in PBS pH 7.2 and then examined by electron microscopy (direct magnification x90000) using 1% sodium silicotungstate negative staining. O, Infectivity (TCID₅₀/0.05 ml); ■, HA activity; △, absorbance at 260 nm; ▲, absorbance at 280 nm: .., buoyant density (g/ml). (a) Empty capsids; (b) standard particles; (c) dense particles.

contaminants and was not characterized further. Empty virus capsids sedimented beneath this component at a density of 1.29 g/ml as shown by electron microscopic examination (Fig. 1). A peak of infectivity and A₂₆₀ containing standard echovirus particles was located at a density of 1.33 g/ml. Another component was detected by infectivity and A₂₆₀ measurements at a density of 1.44 g/ml (Fig. 1) which appeared to be composed of echovirus 11 dense particles. To characterize these denser particles in detail, we compared their various properties with those of standard echovirus 11 particles.
Table 1. Comparison of biological and physical properties of echovirus (HeLa 10) dense and standard particles

<table>
<thead>
<tr>
<th>Property</th>
<th>Standard particles</th>
<th>Dense particles</th>
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<tbody>
<tr>
<td>Buoyant density (g/ml)</td>
<td>1.331 ± 0.004 (11)*</td>
<td>1.438 ± 0.007 (11)</td>
</tr>
<tr>
<td>$A_{260/280}$ ratio</td>
<td>1.71 ± 0.03 (9)</td>
<td>1.69 ± 0.01 (9)</td>
</tr>
<tr>
<td>Specific infectivity (TCID$<em>{50}$/A$</em>{260}$)</td>
<td>20.9 × 10$^6$</td>
<td>17 × 10$^4$</td>
</tr>
<tr>
<td>HA activity (HAU/0.05 ml)</td>
<td>&lt;2</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Neutralizing antibody titre</td>
<td>1600</td>
<td>1600</td>
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</table>

* Assay number.

Antigenic properties were tested using specific rabbit anti-echovirus 11 antisera. The neutralizing antibody titre was similar (Table 1) for both echovirus 11 dense and standard particles. These results suggested that the presence of a denser component was not due to a contamination of our echovirus 11 stock by another virus of higher buoyant density.

RNA to protein ratio estimated from $A_{260/280}$ ratio of virus suspended in PBS pH 7.2 (Yamaguchi-Koll et al., 1975) suggested (Table 1) that dense and standard particles contained similar amounts of RNA and proteins.

For polyacrylamide gel electrophoresis samples were dissociated in 2% SDS and 0.03% β-mercaptoethanol for 1 min in a boiling water-bath, and then placed on 10% polyacrylamide gel containing 0.1% SDS, 0.1% TEMED and 0.1 M phosphate buffer pH 7.2. Electrophoresis was performed in 0.1 M-phosphate–0.1% SDS buffer pH 7.2 at 8 mA/gel for 17 h. After staining (Coomassie Brilliant Blue) the mol. wt. of the polypeptides were estimated by the method of Shapiro et al. (1967). The reference proteins used were: β-galactosidase, bovine serum albumin, ovalbumin and chymotrypsinogen. Polyacrylamide gel electrophoresis of dense and standard particles gave a similar pattern; four polypeptides of mol. wt. 36000, 27000, 23000 and 6500 would correspond respectively to VP1, VP2, VP3 and VP4 of other picornaviruses (Rueckert, 1976) (Fig. 2).

Specific infectivity was estimated as the ratio of infectivity to $A_{260}$ of virus suspended in PBS pH 7.2 (Table 1). The specific infectivity of dense particles was 120 times lower than that of standard particles. This was in agreement with data obtained for other picornavirus dense particles which were also less infective than standard particles (Rowlands et al., 1975; Yamaguchi-Koll et al., 1975). Electron microscopic examination using 1% sodium silicatungstate negative staining of virus suspended in PBS pH 7.2 showed similar features of dense and standard particles which appeared as compact, sphere-like particles (Fig. 1). However, dense particles were stained differently from standard particles (Fig. 1), having brighter circumferences and less granular central parts of the capsids. The average diameter of dense particles (25.28 ± 0.77 nm) was slightly less than that of standard particles (28.10 ± 0.75 nm). Similar differences in size of standard and dense particles were reported for SVDV (Rowlands et al., 1975). Haemagglutinating activity was tested by microtitre method using a 0.6% suspension of human O+ erythrocytes (Cova & Aymard, 1980). No haemagglutinating activity was detected in either dense or standard particles of echovirus 11 (Table 1, Fig. 1).

These results indicated that most of the properties we have tested were similar for dense and standard echovirus 11 particles. Echovirus 11 dense particles seem to be similar to other picornavirus dense particles which are closely related to standard particles. However, echovirus 11 dense particles differ from poliovirus dense particles by their stability. The latter
Fig. 2. Densitometric scanning of the polypeptide components of (a) standard particles and (b) dense echovirus 11 particles. Samples were dissociated in boiling 2% SDS, 0.03% β-mercaptoethanol for 1 min and polypeptides separated by electrophoresis on 10% polyacrylamide gels. Densitometric tracings of Coomassie Blue-stained gels were obtained with a Wernon gel scanner using light of wavelength 580 nm; migration was from left to right. Echovirus 11 coat proteins are: VP1, VP2 and VP3; VP4 migrated out of the gel.
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seem to have a limited stability since they were found in cells only 6 to 12 h after infection and never after 24 h, by which time they were either degraded or converted to another type of particle (Yamaguchi-Koll et al., 1975). In contrast, echovirus 11 dense particles were isolated from extracellular virus at 24 h and 48 h after infection. Echovirus 11 dense particles also differ from other picornavirus dense particles by their relative abundance. The relative amount of poliovirus dense particles calculated from the ratio of $A_{260}$ of dense to standard particles show that dense particles are present as a minor component (10 to 15% of virus harvests) (Yamaguchi-Koll et al., 1975). In contrast, echovirus 11 particles ratio, calculated by the same method, indicated that dense particles represent 50 to 300% of total virus particles. The relative proportion of dense particles was not stable but was always a major component of the virus harvest. We have found this high proportion of dense particles only in echovirus 11 produced in HeLa cells. Haemagglutinating echovirus 11 produced in primary monkey kidney (MK) cells of Maccacus cynomolgus did not contain any detectable levels of dense particles. The absence of dense particles in MK cells and their high level in HeLa cells suggest that the cell system strongly influences the occurrence of dense particles. This was confirmed by cross-passage experiments. When echovirus 11 (HeLa 10) was passaged in MK cells dense particles were not detected.

In summary, echovirus 11 dense particles differ from other picornavirus dense particles in their higher proportion, greater stability and presence in only some cell lines.

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REFERENCES


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