Defective Interfering Particles of Semliki Forest Virus Are Smaller than Particles of Standard Virus

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SUMMARY

By electron microscopy, particles of defective interfering Semliki Forest virus (DI SFV) had a mean diameter of 46.8 nm compared with 55.9 nm for standard virus particles, a decrease of 16%. The difference was confirmed by measurements of the two-dimensional projected areas of DI and standard virus particles. We examined nine different DI virus preparations produced by four to 13 undiluted passages in BHK cells and all were found to contain a majority of the smaller type of particle. Calculation of the absolute number of small particles showed that there were 130 particles per interfering unit measured by the inhibition of virus RNA synthesis. However, a more sensitive assay based on interference with virus protein synthesis gave a particle : interference ratio of 6.5.

Defective interfering (DI) viruses are generated during serial undiluted passage of most, if not all, animal viruses (Huang & Baltimore, 1977) and have a genome which is a deleted form of the infectious (standard) virus genome. Since these viruses are defective and are unable to replicate unaided, propagation is achieved by co-infecting cells with DI and standard virus. The yield from such co-infected cells contains reduced quantities of standard virus, hence the term interference (Holland et al., 1980; Perrault, 1981).

DI viruses have been described for the alphaviruses Sindbis and Semliki Forest (SFV) (Schlesinger et al., 1972; Bruton & Kennedy, 1976) and the genome has been shown to be smaller (often \( < 1 \times 10^5 \)) than that of standard virus (4-2 \( \times 10^6 \)) (Shenk & Stollar, 1972; Eaton & Faulkner, 1973; Weiss & Schlesinger, 1973; Eaton, 1975; Guild & Stollar, 1975; Bruton & Kennedy, 1976; Logan, 1979). The RNA of DI SFV contains no open reading frame \(( \textit{in vivo} \) or \( \textit{in vitro} \) (Bruton et al., 1976; Logan, 1979; A. D. T. Barrett & N. J. Dimmock, unpublished data). Biochemical analysis shows that DI viruses contain the same proteins as standard virus (Bruton et al., 1976; Logan, 1979; A. D. T. Barrett & N. J. Dimmock, unpublished data); thus, it is assumed that DI SFV proteins are synthesized by the standard virus.

Examination of the size of alphavirus DI particles has produced conflicting results. Weiss & Schlesinger (1973) and Logan (1979) reported that DI and standard viruses grown in BHK and mosquito cells respectively were the same size, while Johnston et al. (1975) showed that Sindbis DI virus particles were smaller (37 nm) than standard virus (50 nm) and DI virus preparations with high interfering activity had the greatest numbers of the small particles. These smaller particles were similar in size to morphological variants observed by Brown & Gliedman (1973) during Sindbis virus infection of mosquito cells; however, the latter workers did not determine whether or not these variants were DI particles. Since the standard virus genome represents about 6-3% of the particle mass (Laine et al., 1973) and DI particles have smaller RNA species, this difference should be detected by estimates of particle density. However, the data are conflicting: Shenk & Stollar (1973) and Bruton & Kennedy (1976) have reported that DI virus particles are more dense than standard virus particles while others found no difference in their densities (Weiss & Schlesinger, 1973; Guild & Stollar, 1975; Logan, 1979; Kääriäinen et al., 1981).
In an attempt to clarify these results we have determined the size of DI virus particles from a number of different preparations.

Detailed studies were made of a DI virus preparation which had been propagated for 13 undiluted passages (called DI SFV p13g) in BHK cells as described by Barrett et al. (1981). DI virus and standard virus (Barrett et al., 1981) were concentrated by centrifugation at 90 000 g for 3 h at 4 °C and resuspended in 50 mM-Tris-HCl, 100 mM-NaCl, 1 mM-EDTA, pH 7.4, but were not purified further. Virus was prepared for electron microscopy by placing 10 μl aliquots onto 2% agarose in phosphate-buffered saline (PBS) in the wells of a microtitre plate. A carbon-Formvar-coated copper grid was placed over each drop and the fluid allowed to diffuse into the agarose overnight at 4 °C. The grids were removed, negatively stained with potassium phosphotungstate pH 6.4, and examined for the presence of virus particles in a Philips EM201C electron microscope.

Initial observations showed that both DI and standard virus stocks had a similar morphology but particles in the DI virus preparation appeared smaller (Fig. 1). This was confirmed by measuring the diameters of a number of virus particles from each virus stock using the Mop-Videoplan computerized system for image analysis (Reichert-Jung, Slough, U.K.) and beef catalase crystals for calibration. Standard virus particles had a mean diameter of 55.9 nm in comparison to a smaller mean diameter of 46.8 nm for the DI virus particles (Table 1). Over 95% of the particles in the DI virus preparation were of the smaller type compared with 2% in standard virus (both at 95% confidence limits). The diameters of both DI and standard virus particles had a normal distribution (Fig. 2) confirming that the measurements were significant. These results were extended by using the Mop-Videoplan system to determine the two-dimensional projected areas of the virus particles (Table 1) which were also found to follow a normal distribution. The areas of the DI virus particles (1538 nm²) were 33% smaller than those of the standard virus particles (2308 nm²) which agrees well with the expected value (30%) calculated from the observed reduction in diameter.

Eight other DI virus stocks which had been prepared by between four and 13 undiluted passages in BHK cells were examined by electron microscopy. All contained a majority of small virus particles of diameters similar to those of DI SFV p13g described above. There was some variation in the ratio of small to standard virus particles between stocks but the latter were always a minority (data not shown).

We have previously described a bioassay for measuring interference by DI SFV (the RNA synthesis inhibition assay; Barrett et al., 1981) from which, assuming that interference requires at least one particle per cell, a minimum estimate of the number of biologically active DI virus particles (DI units) can be obtained. This was checked against the number of small particles by determining their concentration by electron microscopy with reference to latex spheres of known concentration; there were approximately 130 small particles for each DI unit. However, we have recently developed an interference assay for DI SFV based upon the inhibition of virus polypeptide synthesis in cells co-infected with standard virus which is 20-fold more sensitive...
Fig. 2. Distribution of diameters of DI and standard SFV particles. ——, DI virus p13g; ——, standard virus. Curves represent normal distribution of diameters of particles.

Table 1. Diameter and area of DI virus and standard SFV particles

<table>
<thead>
<tr>
<th>Virus</th>
<th>Sample size</th>
<th>Mean diameter (nm)</th>
<th>Mean area (nm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>231</td>
<td>55.88 ± 5.96*</td>
<td>2307.56 ± 420.75*</td>
</tr>
<tr>
<td>DI p13g</td>
<td>193</td>
<td>46.79 ± 5.63</td>
<td>1538.14 ± 247.46</td>
</tr>
</tbody>
</table>

* ± 1 standard deviation.

than the RNA synthesis inhibition assay (A. D. T. Barrett & N. J. Dimmock, unpublished data). The greater sensitivity arises presumably from the amplification which results on translation and the superior labelling of polypeptides relative to RNA. On this basis, the DI virus particle : interference ratio is about 6.5 which is of the same order as the particle : p.f.u. ratio of our standard virus.

The data above, showing that SFV DI virus particles are smaller than standard virus particles, are comparable with the data of Johnston et al. (1975) for Sindbis virus but are at variance with the work of others (Weiss & Schlesinger, 1973; Logan, 1979) who find DI and standard virus have the same diameter. However, our DI virus could be partially separated from standard virus by sedimentation through metrizamide (Barrett et al., 1984) thus corroborating their different physical natures. Like Bruton & Kennedy (1976) we found also that DI SFV was denser than standard virus. Now that we know DI particles are smaller than those of standard virus, this observation no longer seems paradoxical for it is perfectly possible to obtain a denser particle even though the amount of RNA/particle is reduced. For the same reason there is now no need to propose that the greater density of DI virus particles is the result of packaging several molecules of DI RNA per particle (Bruton & Kennedy, 1976; Stollar, 1979, 1980). In turn, this is also consistent with the conclusion from the single-hit u.v. inactivation kinetics that there is only one DI RNA molecule per particle (Johnston et al., 1974; Kowal & Stollar, 1980; Barrett et al., 1981).

The assembly of the same structural proteins into two different-sized particles has implications for the understanding of virus particle assembly. In standard SFV, core protein forms a regular geometric structure which appears in DI SFV to have a diameter 16% smaller. If it is assumed that SFV is spherical, the volume of a DI virus particle is 41% less than that of a standard virus particle. To achieve this reduction, either the core subunits are capable of considerable contraction or else can be packed in an alternative array. Clearly, any explanation must await a detailed fine structure analysis.
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REFERENCES


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