Morphological Heterogeneity in Relation to Structural and Functional Properties of Mumps Virus

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SUMMARY

The morphological heterogeneity of the chick embryo-adapted Enders strain of mumps virus was examined in relation to biological and biochemical properties of the virus. The range of virion sizes increased as multiplicity of infection (m.o.i.) increased, with virions of 800 to 1000 nm in diam. occurring in preparations grown at the higher multiplicities. There was no evidence for a distinct class of non-infectious haemagglutinating particles. Dense fractions from isopycnic gradients of virus were enriched in larger virions, but virus RNA was predominantly a single 50S species. Despite evidence that many virions were multiploid, u.v. inactivation kinetics were single-hit, suggesting only a single biologically active genome per virion.

Paramyxoviruses have been observed to display a remarkable morphological heterogeneity, with rounded, filamentous or oblong particles of variable diameter (Valentine & Isaacs, 1957; Waterson et al. 1961; Hosaka et al. 1966). Virion morphology may be related to distinctive biological and biochemical properties. For example, a distinct population of small particles of Sendai virus, containing RNA of smaller size, has defective interfering properties (Kingsbury & Portner, 1970; Kingsbury et al. 1970), and the small non-infectious haemagglutinating particles of Newcastle disease virus (NDV) appear to lack RNA (Dahlberg & Simon, 1969; Roman & Simon, 1976). Electron microscopic studies of negatively stained preparations of the chick embryo-adapted Enders strain of mumps virus (Enders et al. 1946) have shown similar pleomorphism, with virions ranging from 100 up to 800 nm in diam. (Horne & Waterson, 1960; Horne et al. 1960; Finch & Gibbs, 1970). Whereas previous investigations of pleomorphism have emphasized small or defective particles, the present study focused on the morphogenesis and certain biological properties of large mumps virions.

The Enders strain of mumps virus was inoculated into embryonated eggs at varying multiplicities. Virus was isolated from allantoic fluids on discontinuous sucrose gradients. Examination of virus particles with the electron microscope confirmed their pleomorphism with particles ranging up to 1000 nm. The presence of large particles as well as the extent of observed particle size heterogeneity appeared to depend on the m.o.i. (Fig. 1a). This was also true for virus preparations plaque purified on chick embryo fibroblast (CEF) monolayers and was independent of the m.o.i. of earlier passages. Multiple passage of virus did not seem to alter the relationship between size and m.o.i. Virus passed three times at a given m.o.i. exhibited the same degree of size heterogeneity as virus passed once at that m.o.i.

The isopycnic gradient profile of mumps virus when defined by haemagglutinating activity (HA) exhibited a single broad peak with modal density values occurring at 1.176 to 1.187 g/ml, similar to that reported by Huppertz et al. (1977). There was no evidence for a second discrete peak of HA at lower densities, as is the case for egg-grown NDV (Dahlberg & Simon, 1969). A wide range of particle sizes was observed in more dense isopycnic gradient
Fig. 1. Particle size distribution of mumps virus related to (a) multiplicity of infection and (b) particle density. In (a) virus was partially purified on discontinuous 25 to 60% sucrose gradients. In (b) virus was grown at $10^4$ EID$_{50}$ per egg and centrifuged to equilibrium on 25 to 50% sucrose gradients, and individual fractions were examined. Virus samples were applied to 200 mesh collodion and carbon-coated copper grids and negatively stained with 2% phosphotungstate, pH 7.2. The entire grid was scanned under the electron microscope and all virus particles were photographed at a single magnification. Particle areas were calculated using the Hewlett-Packard 9864A digitizer.

fractions (density greater than 1.20 g/ml), but a narrower range of relatively smaller particles was observed in less dense fractions (Fig. 1b). The very large particles (800 to 1000 nm in diam.) appeared predominantly in fractions of density equal to 1.20 g/ml or greater.

When fractions from an isopycnic gradient of virus grown at $10^4$ EID$_{50}$ per egg were analysed simultaneously for HA and infectivity (EID$_{50}$) by methods previously described (McCarthy et al. 1980), the two coincided in the single broad peak in the gradient. Moreover, the ratio of EID$_{50}$ to HA was approximately the same throughout this peak, ranging from $10^4$ to $7.5 \times 10^4$. These are similar to the values obtained by Isaacs & Donald (1955) for unfractionated Enders mumps virus. Thus, we found no evidence for a discrete size class of non-infectious haemagglutinating virus particles or for significant heterogeneity in infectivity as a function of particle density.

The predominance of very large particles in dense gradient fractions suggested that denser particles contain relatively more RNA. Virions from fractions sampled across the gradient peak were dissociated with sodium dodecyl sulphate (SDS) and subjected to gradient centrifugation in the presence of SDS to isolate virus RNA (East & Kingsbury, 1971). The same single 50S species of RNA was observed in all cases; no fractions contained substantial amounts of subgenomic RNA molecules. This is in contrast to results obtained with mumps virus grown in cell culture (East & Kingsbury, 1971) or Sendai virus grown in eggs (Kingsbury & Portner, 1970; Kingsbury et al. 1970). This suggests that some virions are multiploid, i.e. they contain more than one copy of the major 50S RNA. To evaluate
Table 1. Least squares analysis of the u.v. inactivation of gradient fractions of Enders mumps virus*

<table>
<thead>
<tr>
<th>Density (g/ml)</th>
<th>HAU</th>
<th>Slope</th>
<th>Intercept at u.v. dose = zero</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.168</td>
<td>8</td>
<td>-1.18</td>
<td>0.92</td>
</tr>
<tr>
<td>1.173</td>
<td>8</td>
<td>-1.05</td>
<td>0.90</td>
</tr>
<tr>
<td>1.179</td>
<td>16</td>
<td>-0.94</td>
<td>0.90</td>
</tr>
<tr>
<td>1.183</td>
<td>64</td>
<td>-1.05</td>
<td>0.84</td>
</tr>
<tr>
<td>1.203</td>
<td>64</td>
<td>-1.40</td>
<td>1.6</td>
</tr>
<tr>
<td>1.208</td>
<td>64</td>
<td>-1.22</td>
<td>1.1</td>
</tr>
<tr>
<td>1.215</td>
<td>16</td>
<td>-1.02</td>
<td>0.75</td>
</tr>
</tbody>
</table>

Data pooled from all gradient fractions: -1.13, 1.0

* Purified virus from individual isopycnic gradient fractions was diluted 100-fold in Hanks' basic salt solution. Diluted virus was irradiated for varying intervals with 100 ergs/cm²/s u.v. light from a 15 W germicidal lamp. Data were plotted as fraction surviving infectivity on a logarithmic scale versus dose (in ergs) u.v. radiation on a linear scale. The slope of the least squares line equals the change in y in graphical units divided by the change in x in graphical units, and one graphical unit equals 0.5 logarithmic units. The intercept of the line is the fractional infectivity of u.v. dose = zero.

whether or not there are virions with multiple, functionally independent genomes, we determined the u.v. inactivation kinetics of virus infectivity measured by plaque assay on CEF (McCarthy et al. 1980). This method assumes that the virion RNA is the u.v.-sensitive target, so that virus infectivity diminishes with increasing u.v. dose. A plot of the logarithm of the surviving fraction of infectivity versus u.v. dose should extrapolate to give the number of virus genomes when the u.v. dose equals zero (Hiatt, 1964).

The u.v. inactivation of virus from each of seven fractions of an isopycnic density gradient appeared to follow single-hit kinetics and did not support multi-hit inactivation or variation of inactivation kinetics with virion density or size. Least squares analysis was used to generate a best-fit line for each set of inactivation data (Table 1). The predicted y-intercept values, equal to the fraction infectivity when u.v. dose equalled zero, were approx. 1. Least squares lines differed slightly in intercept and in slope, but the variability in the least squares lines apparently derived from the variable range of error (10 to 50%) in plaque assay titrations. A best-fit line was also calculated for the pooled data points. This line had an intercept value equal to 1.

Our results confirm previous observations of mumps virus morphological heterogeneity, demonstrate that the range of particle sizes increases as multiplicity of egg infection increases, and establish that particle size is not genetically controlled. Extraordinarily large particles with diam. from 800 to 1000 nm occur only in preparations of virus grown at higher m.o.i. The tendency to form larger mumps virions must relate directly to the process of virus budding or maturation. Budding paramyxovirus virions vary in size and shape and are often filamentous (Compans et al. 1966; Nakai et al. 1969), in contrast to budding togavirus virions where an icosahedral core becomes enveloped with virus-modified membrane and so defines the boundaries of rounded particles (see Brown et al. 1972; von Bonsdorff & Harrison, 1975). What defines the sizes of budding mumps virions may be a complex function involving cellular regulation of membrane synthesis and replacement as well as the accumulation of virus products and specific interactions between them. The size distribution (Fig. 1a) of mumps virions indicates that a 'preferred' size range exists of approx. 200000 nm² or less, but as the m.o.i. increases, larger particles occur more often, perhaps due to increased quantities of accumulated virus products and/or increased damage to cellular membranes.
Despite evidence for the presence of multiploid virions in mumps virus preparations, u.v. inactivation kinetics suggests that each infectious unit contains only a single biologically active genome. These seemingly paradoxical observations have also arisen in studies of NDV (Granoff, 1959; Dahlberg & Simon, 1969; Kingsbury & Granoff, 1970; Roman & Simon, 1976). Sendai virus reportedly follows multi-hit u.v. inactivation kinetics (Hosaka et al. 1966). The experimental determination of u.v. inactivation of paramyxovirus infectivity is inherently subject to variability and error, since inactivation is determined by the number of u.v. hits per infectious unit, which is several hundred virus particles. Why so many particles are inactive is unknown, but the effect of this phenomenon is that many potential RNA targets cannot be detected. In addition, precise quantification of the number of infectious units is often complicated by error in the method of titration, clumping of virus or other factors.

Failure to demonstrate multi-hit inactivation kinetics in multiploid virions would indicate that many virus genomes are at least initially non-functional, so that an average of only one genome per infectious unit is independently functional. This could be explained by organization of nucleocapsid within the virion. The finding of genetic heterozygotes in NDV populations (Granoff, 1959; Dahlberg & Simon, 1969) suggests that more than one genome can be both replicated and assembled into a mature virion during productive infection. Replication of the genomes in a multiploid virion could depend on the initial transcription of a single independent genome, perhaps to generate sufficient virion transcriptase or other gene products (Bratt & Hightower, 1977).

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REFERENCES


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