Morphology of a Reo-like Virus Isolated from Juvenile American Oysters (Crassostrea virginica)

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SUMMARY

Rotational enhancement of image detail performed on a proposed new serotype of reovirus suggested icosahedral symmetry of T = 3 with some morphological features of members of the Reoviridae family.

A recent report (Meyers, 1979) has characterized a virus (13p2) isolated from hatchery-grown juvenile American oysters (Crassostrea virginica). The virus was psychrophilic and produced syncytial c.p.e. in various fish cell lines. The 13p2 virus was not infectious pancreatic necrosis (IPN) virus of salmonid fish based on its different c.p.e., larger size and failure to neutralize with a polyvalent IPN antiserum. Instead, it was classified as reovirus-like based on size (79 nm), biochemical characteristics and its apparent double-shelled capsid. Untreated virions also showed some capsomere detail on the main capsid layer. These morphological features and stability to pH extremes or high salinities suggested the 13p2 virus was not an orbivirus (Palmer et al. 1977). Alternatively, it was unlike rotavirus with respect to its larger size and lack of a well-defined, smooth outer capsid margin (Palmer et al. 1977). The possibility of the 13p2 virus being a known reovirus contaminant was dismissed on the following criteria: failure to haemagglutinate human type O erythrocytes; negative neutralization tests with types 1 and 3 reovirus antisera; and failure to produce c.p.e. in mammalian and avian cell cultures known to support growth of various reovirus serotypes. In view of this evidence it was proposed that the virus isolate was a new serotype of reovirus of invertebrate or fish origin.

This report is concerned with studies on the fine structure of the 13p2 virus. The objectives were to examine the icosahedral nature of the virus fulfilling the criteria of Caspar & Klug (1962) and Hosaka (1965) and to determine if the new isolate had any other morphological features of reoviruses.

A detailed protocol of virus preparation has been reported elsewhere (Meyers, 1979) Briefly, a pool of supernatant fluids from infected bluegill fry (BF-2) cell cultures exhibiting marked c.p.e. was ultrasonically treated for 20 s and centrifuged at 6975 g for 20 min to remove larger cellular debris. The supernatant was centrifuged at 81 500 g for 3 h in a Spinco SW27 rotor. No attempts were made to further purify the virus. Virus pellets were resuspended in distilled water, adsorbed on to Formvar-coated grids and negatively stained for 1 min in 2% phosphotungstic acid (pH 7.0). Prepared grids were examined in an Hitachi HU-11E electron microscope operated at 100 kV. Rotational enhancement of image detail was done using a pinwheel device similar to that described by Markham et al. (1963) but modified to eliminate the hole produced at the axis of rotation (K. Hirai, unpublished data).

Negatively stained virus particles appeared almost circular or slightly angular depending on the orientation of particle axes. Apparent pentagonal clustering of capsomeres on the closed shell of the main capsid layer was enhanced by an n = 5 rotation (Fig. 1a, b). When rotated on an apparent threefold vertex, six capsomeres were clearly visible on an n = 6 rotation but not when rotated n = 5 (Fig. 1c, d). Stain, which penetrated peripheral capsomeres on several particles, revealed five projections in a 90° quadrant (Fig. 1e) When these particles were rotated on apparent fivefold axes peripheral capsomeres were enhanced

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such that a total of 20 were visible only at a periodicity of $n = 10$. The projections became obscured when particles were rotated $n = 9$ (Fig. 1f, g). Markham et al. (1963) have described the principles necessary for the successful operation of this enhancement method. Briefly, these include accurate centring of the known structures to be enhanced and that $n$ must be equal to the structures' fundamental periodicity or an integral fraction thereof. If done properly the periodicity of the structures will be reinforced resulting in enhancement. The validity of these results can be tested by repeating the rotation at a different periodicity.
defined by \( n - x \), where \( x \) represents a positive integer. If the results were correct structures enhanced at \( n \) should not be clearly defined at \( n - x \). Examples of this can be seen in Fig. 1(c, d, f and g). In addition, if the proper periodicity is achieved and the axis of rotation is the centre of the particle, strong repeats should appear at all radii. This is almost accomplished in Fig. 1(f).

By drawing the apparent surface lattice of the main capsid on to virus particles it also became evident that the capsomeres were arranged in a hexamer–pentamer pattern characteristic of \( T = 3 \) symmetry (Martin et al. 1975) (Fig. 2). In the same negatively stained preparations, occasional particles in various stages of spontaneous degradation were observed. A few of these were without a main capsid layer but instead had six symmetrical peripheral projections apparently protruding from the inner nucleocapsid or vertex capsid layer (Fig. 1h). In addition, fragmented lattice-like arrays of morphological units were observed having a network pattern identical to that visible on the main capsid surfaces of intact virions. Most of these capsomeres were hexagonal and composed of six subunits while only five subunits were discernible in others (Fig. 1i). Subunits appeared to be shared by adjacent morphological units.

There has been considerable controversy concerning the total number of capsomeres contained in the main capsid of reoviruses due to the feature of shared subunits. Subunit sharing does not permit the reliable use of the conventional formula \( [N = 10(n - 1)^2 + 2] \) for computing the number of capsomeres for isometric viruses (Palmer & Martin, 1977). Other difficulties in counting capsomeres have been reported due either to poor capsomere detail (Luftig et al. 1972) or failure to view clearly two vertices of fivefold symmetry on a single capsid surface caused by spacial problems resulting from the large size of individual morphological units (Palmer & Martin, 1977). Large capsomere size has been suggested by recent investigators (Palmer & Martin, 1977) as a criterion for icosahedral symmetry of \( T = 3 \) in reovirus type 3 allowing only a total of 32 capsomeres rather than 92 or 122. The hexamer-pentamer arrangement of capsomeres on the main capsids of \( 13P_2 \) virions would also suggest an icosahedral symmetry of \( T = 3 \) and was consistent with a total of 12 pentamers and 20 hexamers when compared to a similarly orientated model. Although favourably orientated \( 13P_2 \) particles exhibited relatively clear capsomere detail, this was only observed extensively on fivefold axes of symmetry (Fig. 2a). Two axes of fivefold symmetry were never seen on the same closed shell surface of the main capsid layer. The sharing of subunits by adjacent capsomeres on \( 13P_2 \) virus particles resulted in a lattice pattern of subunits around an array of holes. This pattern is consistent with the lattice array reported for reoviruses and differs from other isometric groups of viruses whose morphological units are separate, each with its own subunits (Palmer & Martin, 1977).

Two other morphological features reported by others as occurring in reoviruses were also seen in \( 13P_2 \) particles: the presence of five peripheral projections within a 90° arc around intact particles (Luftig et al. 1972; Palmer & Martin, 1977); and the projection of spikes around the peripheries of vertex capsids stripped of main capsid layers (Luftig et al. 1972; Smith et al. 1969). Among isometric viruses the latter characteristic has been reported in only one other virus besides reovirus. Except for their smaller size (29 nm) untreated kelp fly virus (KFV) particles bear a striking resemblance to the naked cores of reovirus particles (Scotti et al. 1976). The significance of these peripheral spikes and their relationship to total virus structure of the KFV was not known since no outer capsid layer was observed. In reoviruses the peripheral spikes on the nucleocapsid probably exist due to the apparent complex sharing of capsomeres between the vertex and main capsid layers. In reovirus particles treated with chymotrypsin five to nine vertex capsid spikes have been observed and are supposedly arranged on five fold vertices of the icosahedral vertex capsid layer. These reportedly project part way into the main capsid layer (Luftig et al. 1972).
Fig. 2 (a) – for legend see opposite.
Short communications

In conclusion, the 13p₄ virus appears to be an icosahedron with $T = 3$ symmetry and has at least three morphological features unique to the Reoviridae.

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REFERENCES


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