Further Observations on the Ultrastructure of Human Rotavirus

By ERSKINE PALMER* AND MARY LANE MARTIN
Viral Diseases Division, Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia 30333, U.S.A.

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SUMMARY

The inner capsid layer of human rotavirus was found to preferentially break up into large ring-like morphological units. The outer capsid was found to be composed of capsomeres covered by a thin protein or glycoprotein covering. These capsomeres appeared to be broad headed and short stemmed, similar to the type of pin used to mark locations on a map (pushpin).

INTRODUCTION

In previous communications we presented evidence that the inner capsid of human rotavirus is composed of large ring-like morphological units which are, in turn, composed of separate wedge-shaped trimeric subunits (Martin et al., 1975; Palmer et al., 1977). Stannard & Schoub (1977) later suggested that these ring-like structures were moiré-pattern artefacts resulting from superimposition of top and bottom surfaces of the virus. The large units were also considered to be artefacts by Esparza & Gill (1978) and Almeida (1979). The outer capsid layer of rotavirus has been seen by negative-stain electron microscopy as an open lattice following the same symmetry as the inner capsid and located on top of the inner capsid subunits (Stannard & Schoub, 1977; Esparza & Gil, 1978). Freeze-etch electron microscope preparations show that the virus has a smooth surface perforated by holes regularly organized around five- and sixfold axes. The holes correspond one to one with those of the inner capsid.

In this article, we present further evidence that the ring-like structures comprising the inner capsid are true virus structures. Furthermore, we present new findings on the ultrastructure of the outer capsid of human rotavirus.

METHODS

Virus. The Wa strain of human rotavirus adapted to tissue culture by Wyatt et al. (1980) was obtained from G. W. Gary, Jr (Centers for Disease Control, Phoenix, Ariz., U.S.A.) and was propagated in roller bottle MA 104 monkey kidney cell cultures by the method of K. Hancock, G. W. Gary, Jr & E. L. Palmer (unpublished results).

Virus purification. After 4 days incubation at 37 °C, culture media were decanted and centrifuged at low speed in a tabletop centrifuge to remove any cells which had detached from the bottles. Virus in the supernatant was then precipitated with 7-5% polyethylene glycol (PEG). The cell pellet was resuspended in phosphate-buffered saline (PBS) pH 7-2 and added to cells which were scraped from the bottle surfaces with rubber policeman. These were extracted three times with Genesolv-D (trichlorotrifluoroethane) and the aqueous phase then centrifuged for 2 h at 30000 rev/min in a Spinco no. 30 rotor to pellet the virus. Virus was resuspended in PBS and mixed with the PEG precipitate. This mixture was layered on to preformed 30 to 50% glycerol–potassium tartrate density gradients and centrifuged to
equilibrium as described previously (Martin et al., 1975). Virus banded at a density of 1.35 g/ml, and was collected and dialysed against PBS for 48 h to remove density-gradient salts.

Electron microscopy. Purified virus was prepared for electron microscopy by the pseudoreplica technique, and specimens were stained with 0.5% uranyl acetate as described previously (Martin et al., 1975).

RESULTS

Electron microscopic examination of human rotavirus purified by density-gradient centrifugation showed the preparation to contain double-shelled and single-shelled virus particles and lattice-like structures (Fig. 1). About 10% of both types of virus particles were seen to be in various stages of degradation. Single-shelled particles appeared to have large ring-shaped morphological units. When the particles were degraded these units were found in sheets, small groups of two or three or lying free (Fig. 2a, b) and were the predominant unit of disrupted particles. The structures appeared as ring-like units comprised of smaller wedge-shaped subunits. When seen in sheets, the entire lattice was formed by sharing of the wedge-shaped subunits.

Many single-shelled particles had remnants of the outer layer attached to the surface. Fig. 2c, d shows single-shelled particles and attached remnants of outer-shell capsomeres. Those on the particle in Fig. 2c appear broad headed and short stemmed, similar to a pin of the type used to mark locations on a map (pushpin). When viewed from the side the thin pin portion looks to be interlinked with the structure of the inner capsid. The capsomeres are pulling away from the particle. The thick upper part of the capsomeres measures 10 nm in length and is approx. 6 nm wide. The outer layer capsomeres in Fig. 2d are more closely attached to the single-shelled particle and there is a smooth or continuous layer over some of the outer shell layer of capsomeres. This is more clearly seen around the particle in Fig. 2e at a higher magnification. Fig. 2f shows the outer layer capsomeres peeled away from the surface of a single-shelled particle, enabling one to see the three-dimensional aspect of the outer capsomere layer.

The virus preparation also contained many structures which appeared to be comprised of outer shell capsomeres. These are shown in Fig. 3a, b. In Fig. 3a the structure is seen end-on as a short tube of outer shell capsomeres evidently enclosed in the outer protein covering because the capsomeres are not visible. In contrast, Fig. 3b shows the same type of structure with capsomeres visible around the periphery and comprising the inner wall of the tube. The preparation also contained arrays of tubular virus subunits. Some of the tubular forms had about the same diameter as double-shelled particles and showed a well-defined outer edge. These structures are comprised of both outer and inner layer capsomeres (Fig. 3c).

DISCUSSION

The structure of rotavirus has been difficult to define. This is because the large ring-like morphological units on the surface of single-shelled particles are formed by sharing of small wedge-shaped subunits (Martin et al., 1975). These subunits could be considered as capsomeres because, by definition, they are the smallest units of the virus. They have previously been considered as such by Stannard & Schoub (1977). However, definition of vertex points used to define T number is necessary to have a clear understanding of the structure of icosahedral viruses. With viruses such as those of the Herpesviridae distinct columnar capsomeres can be seen aligned in a row between two neighbouring vertex capsomeres. On the other hand, vertex points used to determine T number for mammalian viruses of the Reoviridae are holes, around which are arrayed trimeric subunits. These subunits form large ring-like units, the subunits of which are always shared in groups of two by the
Fig. 1. Survey electron microscopy of human rotavirus from a glycerol-potassium tartrate gradient. The preparation contains double-shelled (DS), single-shelled (SS) and some degraded particles (short arrow). Large ring-shaped morphological units can be seen on the surface of some particles (long arrows). Bar marker represents 100 nm.
Fig. 2. (a) Morphological units of degraded single-shelled particles occurring in groups or lying free. Free-lying units (arrows) are seen to be composed of separate smaller wedge-shaped subunits. (b) The structures show that the large ring-like units form lattices by the sharing of subunits (arrow). (c) Single-shelled particle with remnants of outer shell capsomeres attached. The capsomeres are pulling free and can be seen to be pushpin-shaped. The 'pin' part is interlinked with the inner capsid. (d) Single-shelled particle with remnants of outer shell capsomeres covered with a thin material which is probably protein or glycoprotein (arrow). (e) Higher magnification of a single-shelled particle with about half of its outer shell capsomere layer. Part of the outer layer capsomeres are still covered by the thin outer layer protein (arrow). (f) Disrupting double-shelled particle showing the outer layer capsomeres peeling away from single-shelled particle. All bar markers represent 50 nm.
adjacent capsomeric structure. This sharing of subunits gives the surface of the virus a honeycomb-like appearance. The number of these subunits is a function of T number, but does not fit the formula $N = 10T + 2$ commonly used to calculate capsomere number. For these reasons, we have arbitrarily referred to the large morphological units as capsomeres (Martin et al., 1975; Palmer et al., 1977).

Single-shelled rotavirus particles preferentially break up into the large ring-shaped capsomeres, rather than into the smaller subunits of the capsomere. Sometimes, these capsomeres are in lattice-like arrays, but are frequently seen lying free and appear to be held together by some type of intrasubunit linkage (E. Palmer & M. L. Martin, unpublished)
results). These units have been described as artefacts resulting from superimposition of
Superimposition, however, does not necessarily denote artefactual structure. On the
contrary, superimposition can be an adjunct to the delineation of virus structure since
superimposed images, such as capsomeres from opposite sides of a virus particle are often
more clearly seen than the same single structure in one-sided electron microscope images.
Although superimposition of images probably does enter into the problem of rotavirus
structure, the large units are clearly not artefacts. We have shown that the ring-like units are
true virus structures as initially described by Martin et al. (1975). We agree that the unique
honeycomb-like structure of the inner capsid of rotavirus does require a reassessment of the
terminology applied to the more usual capsid construction (Almeida, 1979), and we recognize
that the wedge-shaped subunits are the basic structural units of the inner capsid. However, the
large ring-shaped morphological units retain their structural integrity when the inner capsid is
degraded. Why these large units do not randomly degrade into smaller subunits remains to be
determined.

Flewett et al. (1974) described the outer layer of double-shelled rotaviruses as having
short, T-shaped capsomeres attached directly to the inner capsid, whereas Esparza & Gil
(1978) considered the outer capsid layer to be more or less planar. More recently, in
micrographs taken by Roseto et al. (1979) of double-shelled particles prepared for electron
microscopy by a freeze-drying technique, the outer layer appeared as a shell with holes that
corresponded one by one with those of the inner capsid. In this study, we have clearly shown
that the first capsid layer of rotavirus particles consists of capsomeres which have a
pushpin-like shape. The smaller ‘pin’ part appears to be interlinked with the inner capsid.
Furthermore, these capsomeres are covered with a thin layer of what is probably protein or
glycoprotein, because it is known that some of the outer shell proteins of rotaviruses are
glycosylated (Rodger et al., 1977). This layer is probably the same as that described as a shell
with small holes on the outer surface of complete rotavirus particles (Roseto et al., 1979) and
could be of major importance in rotavirus serology and immunology because it obscures the
outer layer of capsomeres.

Some of the tubules of lattice arrays of capsomeres had about the same diameter as
double-shelled particles. They consisted of outer layer capsomeres as evidenced by their sharp
edge. The importance of these tubules is not known, but their morphology indicates that they
are virus components which have not assembled into virus particles. Another kind of lattice
array occurs when single-shelled virus is disrupted. These arrays do not have sharp edges and
are irregular in size and shape. That is, they may occur in large sheets, or as small groups, or
even as single ring-like morphological units.

REFERENCES

RODGER, S. M., SCHNAGL, R. D. & HOLMES, I. H. (1977). Further biochemical characterization, including the
ROSETO, A., ESCAIG, J., DELAIN, E., COHEN, J. & SCHERRER, R. (1979). Structure of rotaviruses as studied by the
freeze-drying technique. Institut National de la Sante et de la Recherche Medicale (INSERM) 90, 217–220.
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