

## Ribonucleoprotein-like Structures from Coronavirus Particles

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### SUMMARY

The structure of the ribonucleoprotein (RNP) complex of three coronaviruses was investigated. A single-stranded helix of diam. 14 to 16 nm and up to 320 nm in length was released from disrupted particles of human coronavirus strain 229E and mouse hepatitis virus strain 3 after incubation in mild conditions. The helical complexes appeared to be composed of globular subunits with long axes of 5 to 7 nm surrounding a hollow core of diam. 3 to 4 nm. The complexes were shown to be sensitive to both pancreatic RNase and to pronase. No undegraded internal component was obtained from disrupted avian infectious bronchitis virus particles. We conclude that these structures are RNP complexes. The similarity between these RNPs and those of other large lipid containing RNA viruses is discussed.

Coronaviruses comprise a group of lipid containing RNA viruses that have been classified together mainly on their unique morphology (Tyrrell *et al.* 1975). They appear roughly spherical with diam. ranging from 80 to 120 nm and contain characteristic club-shaped surface projections up to 20 nm in length (McIntosh, 1974). The envelope of the viruses contains lipid and appears to consist of a distinct pair of electron dense shells (McIntosh, 1974). No internal component has been observed in any negatively stained preparations, although a helical component apparently released from virions of the human coronavirus, strain 229E (HCV 229E), has tentatively been identified as part of the ribonucleoprotein (RNP) complex (Kennedy & Johnson-Lussenburg, 1976).

The genomes of coronaviruses consist of a single RNA molecule. These have been shown to be high mol. wt. single-stranded RNA molecules in the cases of HCV 229E (Macnaughton & Madge, 1978), HCV strain OC43 (Tannock & Hierholzer, 1977), avian infectious bronchitis virus (IBV) (Watkins *et al.* 1975; Lomniczi, 1977; Macnaughton & Madge, 1977a; Schochetman *et al.* 1977) and transmissible gastroenteritis virus (TGEV) (Garwes *et al.* 1975). Furthermore, the genomes of HCV 229E and IBV have been shown to contain covalently attached polyadenylic acid sequences (Lomniczi, 1977; Macnaughton & Madge, 1977a, 1978; Schochetman *et al.* 1977). Indirect evidence has been produced suggesting that a polypeptide of about 50000 is associated with the RNA, presumably as a RNP complex, in IBV (Macnaughton *et al.* 1977), mouse hepatitis virus (MHV) (Sturman & Holmes, 1977) and TGEV (Garwes *et al.* 1976).

In this paper we report a morphological investigation into the structure of the internal component of three coronaviruses; namely, HCV 229E, IBV and MHV strain 3. Helical RNP-like complexes were identified associated with virus particles of HCV 229E and MHV3, which were sensitive to pancreatic RNase and to pronase, but no such complexes were obtained from disrupted IBV particles.

HCV 229E was grown in confluent monolayer cultures of continuous MRC cells and then clarified, pelleted and purified on sucrose gradients as previously described (Macnaughton & Madge, 1978). Three IBV strains, Beaudette (IBV 42), Connecticut (IBV 46)

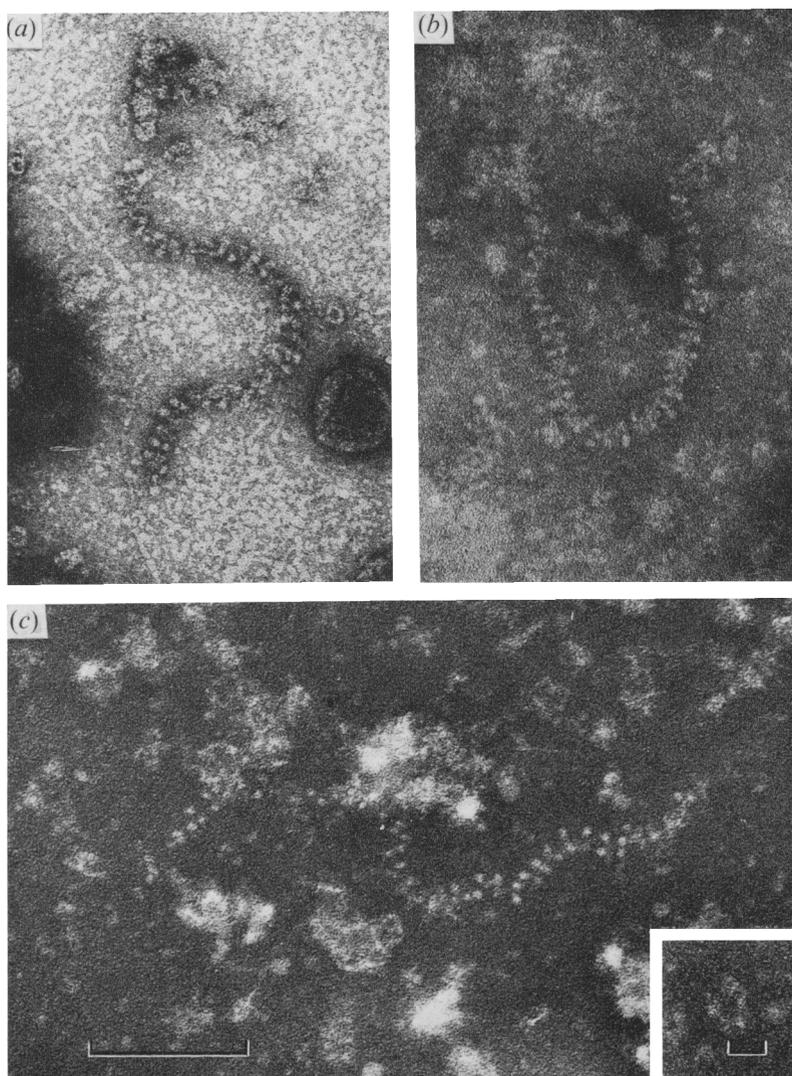


Fig. 1. Preparations of RNP-like structures from purified particles of MHV3 and HCV 229E incubated at 23 °C for 6 to 24 h. (a) HCV 229E, negatively stained with 1% uranyl acetate, pH 4.5. (b) MHV3, negatively stained with 2% potassium phosphotungstate, pH 6.5. (c) HCV 229E, negatively stained with 2% potassium phosphotungstate, pH 6.5. Bar represents 100 nm, except in inset of (c) where it represents 10 nm.

and Massachusetts (IBV 41), were used. They were grown in eggs (Macnaughton & Madge, 1977*b*) or in confluent primary chick kidney cell cultures (Macnaughton & Madge, 1977*a*) and were clarified, pelleted and purified on sucrose gradients (Macnaughton & Madge, 1977*b*). MHV3 was grown in confluent secondary mouse embryonic fibroblasts. The cell monolayers were infected at an input multiplicity of 0.1 infectious particles per cell and, following an adsorption period of 1.5 h at 37 °C, were incubated for 72 h at 37 °C in Eagle's MEM with 2% foetal calf serum. The virus was clarified, pelleted and purified on sucrose gradients as described above for HCV 229E and IBV.

Virus particles of density 1.18 g/ml were examined after negative staining by electron microscopy. Typical coronavirus particles were observed, with coronas of surface projections and intact membranes, as previously described (Macnaughton & Madge, 1977*b*; Macnaughton & Madge, 1978). Virus preparations were treated with detergents, such as Nonidet P-40 and sodium dodecyl sulphate, in order to disrupt the membranes and to release the internal components. The coronavirus membranes were disrupted, but as in other studies, no recognizable RNP complexes were seen (Berry *et al.* 1964; Kaye *et al.* 1970; Kennedy & Johnson-Lussenburg, 1976; Macnaughton *et al.* 1977).

Because the internal components of coronaviruses appeared to be so fragile, gentler procedures for their isolation were then used. Purified virus preparations from sucrose gradient fractions, containing Dulbecco's phosphate buffered saline 'A' pH 7.2, were incubated for between 6 and 24 h at 23 °C and were then negatively stained with 2% (w/v) potassium phosphotungstate, pH 6.5, or 1% (w/v) uranyl acetate, pH 4.5, and examined in a Philips EM 300 electron microscope. Coronaviruses incubated at 37 °C were completely disrupted. Fig. 1 shows electron micrographs of RNP-like structures released from MHV3 (Fig. 1*b*) and HCV 229E (Fig. 1*a, c*) after incubation at 23 °C. The number of complexes obtained varied greatly but usually about one was observed for every ten virus particles. RNP-like structures isolated from HCV 229E and MHV3 particles appeared identical to each other. Different negative staining procedures, with uranyl acetate (Fig. 1*a*) and potassium phosphotungstate (Fig. 1*c*), produced little difference in the morphology of HCV 229E. However, no complexes were obtained using either negative stain from any of the IBV strains used—Beaudette, Connecticut or Massachusetts—although amorphous structures, similar to those described by Kennedy & Johnson-Lussenburg (1976) for HCV 229E, were occasionally observed in disrupted IBV particles.

The RNP-like structures of HCV 229E and MHV3 appeared similar to those of other large lipid containing RNA viruses, such as orthomyxoviruses and paramyxoviruses (Compans & Chopin, 1973). The complexes from both coronaviruses appeared as helical structures of diam. 14 to 16 nm. Small circles were seen in some preparations, apparently caused by fragmentation of the helical structures (Pons *et al.* 1969). These circular structures appear to consist of a single turn of the helix surrounding a hollow core of diam. 3 to 4 nm. Furthermore, the helical strands seem to be composed of globular subunits with their long axes, of length 5 to 7 nm, normal to the axis of the helix. About five subunits per turn of the helix could be observed in some electron micrographs (Fig. 1*c*, inset). The mol. wt. of these apparently globular subunits was estimated as described by Green (1969) to be between 50000 and 70000.

To demonstrate that the observed structures from HCV 229E and MHV3 indeed contained RNA and protein, they were incubated with pancreatic RNase (100 µg/ml) and pronase (400 µg/ml) for 30 min at 23 °C using latex beads as an internal standard. The percentage decrease in the number of RNP-like complexes after incubation is shown in Table 1. All the complexes were disrupted by pronase and over 80% of them by pancreatic RNase. Under the same conditions of RNase digestion, less than 5% of HCV 229E RNA, extracted with proteinase K (Macnaughton & Madge, 1978), resisted pancreatic RNase. These results suggest that the RNP-like complexes contain RNA and protein, and that some of the RNA may be protected by protein from RNase digestion.

We conclude that the observed structures, released from HCV 229E and MHV3 particles after gentle incubation, are in fact RNP complexes. They comprise a single-stranded helix of diam. 14 to 16 nm, which is itself composed of globular subunits with long axes of 5 to 7 nm surrounding a hollow core of diam. 3 to 4 nm. We may have failed to visualize

Table 1. Digestion of coronavirus RNP-like structures with pancreatic RNase and pronase

Coronavirus RNP-like species	Enzyme incubation conditions*	Number of RNP-like structures $\times 10^{-8}$ /ml		Percentage of RNP-like particles undigested
		Before incubation	After incubation	
HCV 229E	No enzyme	15	15	100
	Pancreatic RNase (100 $\mu$ g/ml)	43	6.4	15
	Pronase (400 $\mu$ g/ml)	31	0	0
MHV <sub>3</sub>	No enzyme	4.5	4.5	100
	Pancreatic RNase (100 $\mu$ g/ml)	5.2	1.0	19
	Pronase (400 $\mu$ g/ml)	3.4	0	0

\* Samples were incubated at 23 °C for 30 min with pancreatic RNase and pronase added where indicated.

the RNP in IBV strains grown in eggs or primary kidney cell cultures because it is particularly unstable and not because it is absent. However, we have observed circular structures in IBV preparations of the same size and diameter as those from HCV 229E and MHV<sub>3</sub>. These structures may be produced by fragmentation of IBV RNP complexes. The internal components of HCV 229E and MHV<sub>3</sub> appear to be morphologically similar to those of other large lipid-containing RNA viruses, although they are much more fragile. The polypeptide component of the RNPs of orthomyxoviruses and paramyxoviruses is about 60000 (Lenard & Compans, 1974), which is similar to that of coronaviruses (Garwes *et al.* 1976; Macnaughton *et al.* 1977; Sturman & Holmes, 1977). Our estimation of the mol. wt. of the RNP subunits of HCV 229E and MHV<sub>3</sub> is between 50000 and 70000, which is similar to our value of 50000 for the mol. wt. of the internal component polypeptide of these viruses, determined by polyacrylamide gel electrophoresis (M. R. Macnaughton, unpublished data). However, although the RNA genome of coronaviruses is about the same size as that of paramyxoviruses such as Newcastle disease virus (Matthews, 1975), it is unlike them in being polyadenylated (Lomniczi, 1977; Macnaughton & Madge, 1977*a*, 1978; Schochetman *et al.* 1977). Experiments are in progress to separate the RNPs of HCV 229E and MHV<sub>3</sub> from the disrupted virus particles in order to analyse further their biochemical structure and to relate their structure to that of other large lipid containing RNA viruses.

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