Characterization of Pig Rotavirus RNA

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SUMMARY

Pig rotavirus was purified from faeces. The RNA from this virus was resistant to pancreatic ribonuclease, indicating that it is double-stranded. When electrophoresed on polyacrylamide-agarose gels, pig rotavirus RNA migrated as 9 bands comprised of 11 or 12 RNA segments with a total mol. wt. of approx. $11 \times 10^6$. Co-electrophoresis experiments revealed that the RNAs from the pig virus and two isolates of the calf rotavirus were indistinguishable.

Rotaviruses have been associated with diarrhoea in a variety of animal species including calves (Mebus et al. 1969), piglets (Woode & Bridger, 1975), foals (Flewett, Bryden & Davies, 1975), lambs (Snodgrass et al. 1976) and humans infants (Bishop et al. 1974). Biochemical characterization has revealed that the calf rotavirus RNA is double-stranded and exists as 11 or 12 segments with mol. wt. in the range $2.2 \times 10^6$ to $0.2 \times 10^6$ (Newman et al. 1975; Rodger, Schnagl & Holmes, 1975). In the experiments reported in this paper we have characterized the RNA of a pig rotavirus and compared it with that of two isolates of the calf rotavirus.

Diarrhoeic pig faeces (20 ml), which had been shown to contain rotavirus by electron microscopy, were diluted fivefold with 0.02 M-tris-HCl, pH 7.5 (tris buffer), mixed with 50 ml of Arcton 113 (Arklone; ICI Ltd) and sonicated for 10 to 15 s. After centrifugation at 1000 g for 5 min the Arcton phase was re-extracted with 50 ml of tris buffer and the aqueous phase pooled and re-extracted with a half volume of fresh Arcton 113. The suspension was clarified by centrifugation at 2500 g for 20 min and the supernatant fluid centrifuged at 75000 g for 1 h. The pellet obtained was resuspended in tris buffer and layered on top of 10 ml of 40 % (w/v) sucrose. After centrifugation at 50000 g for 2 h the resulting pellet was resuspended and incubated for 0.5 h at 37 °C with deoxyribonuclease (20 μg/ml; Sigma Chemical Co.) and pancreatic ribonuclease (10 μg/ml; Sigma Chemical Co.) in tris buffer containing 0.1 M-NaCl and 0.0025 M-MgSO₄. Samples (2 ml) were layered on to 11 ml pre-formed CsCl gradients (density range 1.10 to 1.45 g/ml) and the gradients centrifuged at 110000 g for 3 h in a swing-out rotor. Virus bands with density 1.35 to 1.37 g/ml were removed with a Pasteur pipette, diluted with tris buffer and the virus collected as a pellet by centrifuging at 75000 g for 1 h. All purification steps were carried out at 4 °C.

The calf rotaviruses, namely the Northern Ireland (McNulty, Allan & McFerran, 1976) and Nebraska (Mebus et al. 1969) isolates were grown in Madin-Darby bovine kidney cells (American type culture collection number 22). Infected cell cultures in 40 oz roller bottles were maintained in Eagle's BHK medium (Wellcome Reagents Ltd) containing 2 % foetal calf serum for 24 to 48 h at 37 °C. After freezing and thawing the contents of the roller bottles, the resulting cell lysate was sonicated for 10 to 15 s and virus was purified by a method similar to that used for pig rotavirus. This involved differential centrifugation, Arcton 113 extraction, sedimentation through 40 % (w/v) sucrose, and density gradient centrifugation in CsCl. Calf rotaviruses banded at a density of 1.35 to 1.37 g/ml. Reovirus type 1, grown in 40 oz roller bottle cultures of Vero cells, was purified in an identical manner to that used for the calf rotaviruses.
Fig. 1. Electrophoresis of RNA on 2.5% (w/v) polyacrylamide-agarose gels. Ethidium bromide-stained RNA was visualized under a u.v. lamp. (a) Left: pig rotavirus RNA electrophoresed for 4 h. Nine bands of RNA indicated by arrows. Right: pig rotavirus and reovirus type 1 RNAs co-electrophoresed for 4 h. Letters indicate the position of the reovirus RNA mol. wt. markers. (b) Co-electrophoresis (4 h) of pig and calf rotavirus RNAs. (c) Co-electrophoresis (5 h) of Northern Ireland and Nebraska calf rotavirus RNAs.

Virus particles were disrupted by treatment with a solution containing 3 M-urea, 1% (w/v) SDS and 10% (w/v) sucrose at 37 °C for 0.5 h. Samples (10 to 20 µl) of this mixture were electrophoresed on cylindrical (8 cm) polyacrylamide-agarose gels containing 2.5% (w/v) acrylamide. Gels were prepared as described by Clements & Martin (1971). Samples of RNA were electrophoresed at 6 mA/gel for 4 to 5 h. RNA bands were stained with ethidium bromide (100 µg/ml) in distilled water for 0.5 h and visualized using a u.v. lamp. Photographs were taken with a camera equipped with an orange filter. Alternatively RNA was stained with methylene blue as described by Shatkin, Sipe & Loh (1968) and scanned at 590 nm using a Joyce Loebl Chromoscan.

Examination in the electron microscope confirmed that the purified pig and calf rotaviruses were largely free of contaminating material. Purified virions were of the 60 nm single capsid type described by Holmes et al. (1975). RNA extracted from pig rotavirus preparations, using phenol equilibrated with 0.1 M-sodium acetate-acetic acid, pH 5.0, (acetate) buffer
containing 0·1 % (w/v) SDS, and purified by gel filtration through Sephadex G25, was found to be resistant to treatment with pancreatic ribonuclease (10 μg/ml at 37 °C for 0·5 h in acetate buffer). This indicated that the RNA of the pig rotavirus was double-stranded.

Electrophoresis of pig rotavirus RNA (Fig. 1a) showed that the double-stranded genome was segmented and migrated as 9 bands. Band number 7, which was proportionally more intense in relation to its size, probably consisted of several RNA species with the same electrophoretic mobility. Co-electrophoresis of pig rotavirus RNA with reovirus type I RNA allowed the mol. wt. of the pig rotavirus RNA segments to be estimated (Fig. 2) by comparison with the mol. wt. 2·5 (L), 1·4 (M) and 0·8 (S₂) x 10⁶ (Shatkin et al. 1968) of the reovirus RNA segments. The mol. wt. estimates of the pig rotavirus RNA segments thus obtained ranged from 2·1 x 10⁶ to 0·3 x 10⁶. Experiments in which Northern Ireland calf rotavirus RNA was co-electrophoresed with pig rotavirus RNA (Fig. 1b) revealed no differences in the number or size of the RNA segments of the pig and calf viruses. Similarly no differences were detected between the RNA segments of the two calf rotavirus isolates (Fig. 1c).

Extinction profiles obtained by scanning methylene blue-stained gels (Fig. 2) were used in the calculation of the molar ratios of each of the nine RNA peaks. All peaks except number 7 appeared to represent RNA molecules present in equimolar amounts, whereas peak number 7 comprised 3 or 4 RNA species of similar mol. wt. Thus, the pig rotavirus RNA consisted of 11 or 12 segments with a total mol. wt. of approx. 11 x 10⁶.

This is the first report of the characterization of RNA from a pig rotavirus. The RNA was double-stranded and migrated as nine bands when electrophoresed on 2·5 % (w/v) polyacrylamide-agarose gels. The mol. wt. estimates of these RNA segments were in close agreement with those reported by others for calf rotavirus RNA (Newman et al. 1975;
Rodger et al. (1975). Co-electrophoresis experiments demonstrated that the RNAs from the pig rotavirus and two isolates of the calf virus were indistinguishable. The close similarity between pig and calf rotavirus RNAs was not surprising in view of the antigenic similarities which exist within the rotavirus group (Kapikian et al. 1974; Woode & Bridger, 1975; Snodgrass et al. 1976).

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REFERENCES


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