Identification of heat-dissociable RNA complexes in two porcine coronaviruses

The coronavirus genome has been shown to comprise single-stranded RNA. Examination of the viral nucleic acid synthesised by pig kidney cells infected with transmissible gastroenteritis virus (TGEV) suggested that several molecular species, ranging in size between 18 and 28S, were involved in the viral replicative cycle; similarly Tannock found a wide variation in the size of RNA molecules extracted from avian infectious bronchitis virus (IBV) by a phenol–sodium dodecyl sulphate (SDS) method. Extraction of IBV RNA by 1% SDS at 60°C has, however, revealed a single component of molecular weight 9×10^6 corresponding to 60S by electrophoresis through 2.2% polyacrylamide gels.

We have examined the RNA extracted from purified preparations of TGEV and a second porcine coronavirus —haemagglutinating encephalomyelitis virus (HEV)— and have found a 60–70S RNA component which dissociates into 35S and 4S material on heating above 60°C in a way that closely resembles the genome of the oncogenic RNA viruses.

We had observed that treatment of purified TGEV with 1% SDS at 20°C disrupted the virions and liberated a high molecular weight complex containing the RNA. On the assumption that this complex might comprise the hitherto undetected ribonucleoprotein, we extracted the material from TGEV preparations radioactively labelled with 3H-uridine or with 3H-leucine to determine which structural polypeptide was associated with the complex. As is shown in Fig. 1, however, the fast moving RNA complex has no detectable protein associated with it, while polyacrylamide gel analysis of the radioactivity remaining near the top of the complex.

Fig. 1 Rate zonal sedimentation of TGEV after treatment with 1% SDS at 20°C. TGEV was grown in secondary pig thyroid cell (APT/2) cultures in the presence of 5-H-uridine (●) or 4,5-3H-leucine (○) and purified by sucrose gradient centrifugation. The gradients were fractionated by siphon and total radioactivity was determined for each fraction.
the gradient demonstrated the presence of viral structural polypeptides.

Extraction of RNA from 3H-uridine-labelled TGEV by 1% SDS at 20°C followed by electrophoresis through gels containing 2% polyacrylamide and 0.6% agarose revealed a homogeneous band of high molecular weight RNA and a very small amount of radioactivity in the area of the marker dye, corresponding to approximately 4–7S (Fig. 2a).

Similar extractions were performed on replicate virus samples using 1% SDS at 40, 60, 80 and 100°C and the released RNA was electrophoresed as before. The electropherograms illustrated in Fig. b–e showed that the mobility of the major RNA band increased slightly as the extraction temperature was raised to 60°C and, while the homogeneity of the leading edge of the band decreased somewhat, there was no obvious change in the 4S–7S region. As the temperature was increased through 80 to 100°C, however, the radioactivity became associated with a broader band of RNA, whose mobility was at least twice that of the RNA extracted at lower temperatures, and there was an increase in the amount of RNA in the 4S region of the gel.

This apparent “melting” of the high molecular weight complex into smaller components with the liberation of 4S RNA at temperatures above 60°C closely resembles the findings for the oncogenic RNA viruses. To determine the size of the coronaviral RNA components and their similarity to oncornaviral RNA, we compared the mobilities of RNA extracted from TGEV and HEV at 20 and 100°C with those of purified Rous sarcoma virus (RSV) RNA held at the same temperatures. Figure 3a–f shows that, by this method, the RNA complexes extracted from the two coronaviruses are indistinguishable in size from the 60–70S component of RSV RNA and that, after heating, the TGEV and HEV RNA components are comparable in size to the RSV 35S RNA. There seems to be more heterogeneity in the coronaviral 35S RNA band than in the RSV equivalent and this holds true regardless of the time of the labelling period. Comparison of RNA extracted from TGEV labelled with 3H-uridine 0–4, 4–8, 8–12, 12–16 or 0–20 h after infection shows that, although the total radioactivity incorporated varied with the time of labelling period, the overall shape of the RNA curves after extraction at 20 and 100°C were similar. This difference in heterogeneity may be due partly to the fact that we were comparing extracts from whole HEV and TGEV with purified 60–70S RNA from RSV, but it may also be caused by partial degradation of the RNA within the virion. That this undoubtedly occurs is demonstrated by the electropherograms illustrated in Fig. 3g–h, which were all derived...
from TGEV that had been held at 4 °C for 20 d before extraction at 2 and 100 °C in 1% SDS. The 60–70S complex seems to be intact, but, on melting, the complex liberates only small fragments of RNA of approximately 4S (Fig. 3h). This suggests that the virus preparations have an associated ribonuclease capable of producing breaks in the 3S strand while they are complexed in the 60–70S form. Whether the large amount of 4S RNA detected in all HEV preparations so far examined (Fig. 3e–f) represents degraded viral RNA or host tRNA associated with the virions is not known.

Our inability to detect protein in the 60–70S RNA complex from TGEV does not exclude the possibility that there is a very small amount that is dissociating from the RNA at elevated temperatures in the presence of SDS. The similarity of behaviour and size between the coronaviral RNA and the RNA from oncornaviruses, together with the liberation of 4S RNA on melting suggests strongly that the TGEV and HEV 60–70S complex is held together by RNA–RNA interactions as is the RNA from oncornaviruses. We hope to characterise further the 60–70S complex and determine whether the viral 4S component is in fact host tRNA. Although the replication of these two groups of viruses is fundamentally different, the coronaviruses being entirely cytoplasmic in contrast to the essential nuclear phase of the oncornaviruses, a similarity in the structure of the genomes of the two groups raises interesting implications for the phylogeny of the RNA tumour viruses.

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Presence of factor VIII-related antigen in blood platelets of patients with Von Willebrand's disease

Von Willebrand's disease (VWD) is an autosomally inherited disorder characterised by low factor VIII activity (antihaemophilic factor, AHF), prolonged bleeding time, reduced retention of platelets in a glass bead column and abnormal distocetin-induced platelet aggregation. The prolonged bleeding time in VWD has been attributed to the absence of a plasma factor, the von Willebrand factor (VWF), as shown by a correction of the bleeding time after air drying of a drop of the same platelet suspension by a previously described indirect immunofluorescent technique. The onether hand, the localisation of F VIII-RA through-out the megakaryocytic cytoplasm is in agreement with its localisation inside the platelet. Cross-immunoelectrophoretic analysis revealed an electrophoretic mobility comparable with that of plasma F VIII-RA.

Howard et al. quantitated F VIII-RA on intact washed normal platelets and concluded that an F VIII-RA was firmly bound to the membrane fraction. A similar conclusion was reached by Bloom using an immunofluorescent technique. Suspensions of intact washed platelets were incubated with antifactor VIII in suspension, washed again, and incubated with fluorescein isothiocyanate-labelled horse anti-rabbit globulin (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam). A drop of this suspension was studied under the fluorescence microscope. Most platelets were unstained, whereas a vivid granular staining was obtained after disruption of membranes by air drying of a drop of the same platelet suspension on a glass slide. The specificity tests have been described in detail elsewhere. Staining with normal rabbit serum instead of with antifactor VIII was used as a control. The difference between staining in suspension or staining after air drying suggests that F VIII-RA is present inside the platelets.

In agreement with the results of Howard and Nachman and Jaffe reported the presence of factor VIII activity in platelets of patients with VWD. This F VIII-RA supported aggregation induced by ristocetin in a washed platelet system, a property of factor VIII which has been attributed to VWF activity.

Human blood platelets were washed according to the method of Karpatin. ACD-blood was centrifuged (10 min, 200g, 20 °C). Platelet rich plasma was diluted in 5 volumes of Krebs-Ringer buffer (pH 7.4) containing 9 mM Na2-EDTA. After centrifugation (15 min, 1,000g, 4 °C), the platelets were washed once in Krebs-Ringer buffer containing 9 mM Na2-EDTA and twice in Krebs-Ringer buffer containing 1 mM Na2-EDTA. The platelet pellet was finally resuspended in Krebs-Ringer buffer containing 30 mM glucose and 1 mM 2-epsilon caproic acid. In the final washing fluid the protein content was lower than 50 µg ml−1 (ref. 11), and the concentration of F VIII-RA, measured by electroimmunodiffusion was below 0.05 U ml−1 (ref. 12). 1 U F VIII-RA was defined as the amount present in 1 ml pooled normal plasma prepared from 40 healthy subjects.

In the final suspension (1 × 1010–3 × 1010 platelets ml−1) the platelets were disrupted by freezing and thawing (four times) followed by centrifugation (60 min, 30,000g, 4 °C). Ten suspensions of normal human platelets were tested. The concentration of F VIII-RA detected in the supernatant was 0.15 U mg−1 platelet protein (range 0.11–0.25) or 43 U 1011 platelets (range 19–94). Expressed per platelet (range 8–94) was defined as the amount present in 1 ml of the platelet suspension (8 × 1010–1510) (ref. 13). The concentration of F VIII-RA was 60 times (range 21–156) higher than that in plasma. Similar values were reported by Nachman and Jaffe.

Platelet F VIII-RA showed a reaction of identity with plasma F VIII-RA, when tested in immunodiffusion using a rabbit anti-factor VIII serum and the antiserum raised against the low ionic strength components (ref. 15 and B.N.B., J. van Mourik, S. de G., J.M.H.-H. and J.J.S., unpublished). Cross-immuno-electrophoretic analysis revealed an electrophoretic mobility comparable with that of plasma F VIII-RA.