THE CHARACTERISATION OF THE VIRION RNA OF AVIAN INFECTIOUS BRONCHITIS VIRUS

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1. Introduction

Avian Infectious Bronchitis Virus (IBV) is a member of the group of enveloped viruses called coronaviruses [1]. The particles of IBV are pleomorphic and range in diameter from 80–120 nm. They typically contain a corona of widely-spaced club-like surface projections up to 20 nm in length [2,3].

Previous reports have shown that the nucleic acid of IBV is RNA [4,5] although there is some discrepancy as to the size of this RNA. Tannock [4] analysed phenol-extracted IBV RNA and showed it to consist of extremely heterogeneous RNA fragments, while Watkins et al. [5] have shown that the IBV-genome extracted with 1% SDS at 60°C is a single large mol. wt (9.0×10^6) RNA species. Another report [6] has shown that the coronaviruses, transmissible gastroenteritis virus and haemagglutinating encephalomyelitis virus also contain single large molecular weight RNA genomes. The present report describes a method of isolation of IBV-RNA, using proteinase K, that reveals a single single-stranded high mol. wt (approx. 8.5×10^6) RNA species.

The virion RNAs of a number of RNA viruses have been shown to contain a poly(A) segment linked covalently to the 3′-terminus of the RNA, although so far no reports have indicated the presence of such sequences in the genomes of coronaviruses. However, in this paper, we show that the virion RNA of IBV is polyadenylated.

2. Materials and methods

IBV-Beaudette was grown in confluent primary chick-kidney cell (CKC) cultures [7]. [^{3}H]Uridine (10 μCi/ml) or [^{3}H]adenosine (10 μCi/ml) were added just after infection and the virus harvested after 25 h.

After three freeze–thaw-cycles of the cell cultures, the suspension was clarified by centrifugation at $2000 \times g$ for 30 min at 4°C and then pelleted at $75\,000 \times g$ for 1 h at 4°C. The pellet was resuspended in 1 ml PBSA (Dulbecco's phosphate buffered saline 'A') and overlaid on to a 23 ml linear 25–55% (w/w) sucrose density-gradient in PBSA and centrifuged for 16 h at $90\,000 \times g$. Aliquots (1 ml) were collected and the fractions containing virus were located by assay of radioactivity [9].

The RNA was extracted using proteinase K [8], purified on cellulose CF 11 columns [9] and run on 2.2% polyacrylamide gels supported by 0.5% agarose [9]. Oligo(dT)-cellulose chromatography was performed as previously described [10]. Nuclease digestion of IBV-RNA was done at 37°C for 30 min in a solution containing 200 mM NaCl, 20 mM Tris–HCl, pH 7.5, 10 μg/ml pancreatic ribonuclease A and 30 units/ml T_1 ribonuclease.

3. Results

Virus banded at 1.18 g/cm^3 in sucrose-gradients and when analysed by electron microscopy was found to exist as typical coronavirus particles [2].

RNA extracted from IBV-particles, with proteinase K, was run on 2.2% polyacrylamide gels. Figure 1 shows a typical profile with the RNA appearing polyacrylamide gels. Figure 1 shows a typical profile with the RNA appearing as a single peak at 58 S, corresponding to approx. mol. wt 8.5×10^6. This RNA

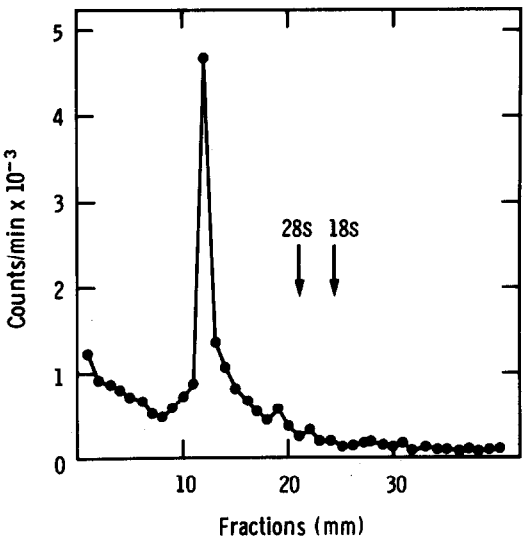

Fig.1. Electrophoresis on a 2.2% polyacrylamide gel of IBV-virion RNA labelled with [^{3}H]uridine. The arrows indicate the positions of CKC rRNA.

was treated with 10% formaldehyde for 10 min at 67°C [8] but the electrophoretic mobility of the RNA on polyacrylamide gels was unchanged. Since this treatment removes all hydrogen bonding in RNA [8] the result indicates that there is little or no secondary structure in the IBV genome. IBV-RNA was almost totally digested with ribonuclease treatment (table 1); thus it was concluded that it is single-stranded.

Table 2
The binding of various polyribonucleotides to oligo(dT)-cellulose columns

Polyribonucleotide	Percentage binding
Poly(A)	100
IBV RNA	27[a]
CKC rRNA	2
Poly(U)	0

[a] Average of 5 determinations

To determine whether or not IBV-RNA is polyadenylated, [^{3}H]uridine labelled IBV-RNA was passed through an oligo(dT)-cellulose column. About 27% of the RNA was bound and eluted from the column (table 2). This is a minimum estimate of binding as a further 4–7% of the RNA not binding to the oligo(dT)-cellulose column could be bound and eluted during a second cycle of chromatography. Poly(A) bound 100% to the column as expected and poly(U) and CKC rRNA, which would not be expected to contain any poly(A) sequences, bound 0% and 2% respectively to the oligo(dT)-cellulose column. This result suggests that the IBV RNA contains a covalently bound poly(A) sequence.

Digestion of IBV-virion RNA with pancreatic ribonuclease A and T_1 ribonuclease, which do not destroy poly(A) tracts, was done in order to confirm that the virion RNA is polyadenylated. Table 1 shows that [^{3}H]adenosine labelled RNA was about 1.5%

Table 1
Binding of labelled IBV-RNA to oligo(dT)-cellulose columns after digestion with nucleases[a]

Labelled RNA species	Initial cpm	Binding to oligo(dT)-cellulose	
		(cpm)	(%)
[^{3}H]Adenosine labelled IBV-RNA	41 761	531	1.2
	18 747	296	1.5
	23 412	289	1.8
[^{3}H]Uridine labelled IBV-RNA	27 669	39	0.1
	35 693	46	0.1
	51 871	41	0.1

[a] Digestion was at 37°C for 30 min in a solution containing 200 mM NaCl, 20 mM Tris–HCl, pH 7.5, 10 μg/ml pancreatic ribonuclease A and 30 units/ml T_1 ribonuclease

resistant to such digestion and this RNA was polyadenylated as it could be recovered from oligo(dT)-cellulose columns. On the other hand, [^{3}H]uridine-labelled IBV-virion RNA was totally digested under the same conditions and no labelled poly(A) could be recovered after binding to oligo(dT)-cellulose.

4. Discussion

The results confirm a previous result which shows that the size of the IBV-genome as estimated on polyacrylamide gels corresponds to 58 S [5]. Treatment of IBV-RNA with formaldehyde, which is known to remove RNA secondary structure [8], produced little difference in the migration of the RNA on polyacrylamide gels. Furthermore, essentially all the IBV-RNA was digested with ribonuclease. These results show that there is little, if any, secondary structure or double-strandedness in the IBV-genome. Therefore, we conclude that the genome of IBV is a single single-stranded RNA molecule approx. mol. wt 8.5×10^6.

Furthermore, we have shown by oligo(dT)-cellulose chromatography and resistance to ribonuclease digestion, that IBV-RNA is polyadenylated. This result implies that IBV is a positively stranded RNA virus. Further experiments are in progress to see if these results are common to other coronaviruses.

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