Archives of Virology 55, 47—54 (1977)

Archives of Virology


# The Polypeptide Composition of Avian Infectious Bronchitis Virus Particles

By

M. R. Macnaughton and M. Hilary Madge

Division of Communicable Diseases, Clinical Research Centre, Harrow, Middlesex, England

With 4 Figures

Accepted April 5, 1977

## Summary

Egg grown avian infectious bronchitis virus (IBV) centrifuged on sucrose density gradients was found to consist of a major virus peak of density 1.17 to 1.18 g/cm$^3$ and occasionally two minor virus peaks of density 1.21 to 1.22 g/cm$^3$ and 1.13 g/cm$^3$. Three different IBV strains were examined and no morphological differences were detected between virus particles of different densities or from different strains. The polypeptides of the different density virus particles from the three IBV strains were analysed on polyacrylamide gels. In all cases 7 polypeptides were observed, although there were differences in the proportions of these polypeptides in particles of different densities and those from the different strains. The polypeptides have been called VP1 (molecular weight 130,000), VP2 (105,000), VP3 (97,000), VP4 (81,000), VP5 (74,000), VP6 (51,000) and VP7 (33,000). Additional polypeptides were produced if slightly harsher treatments were used.

## Introduction

Avian infectious bronchitis virus (IBV) is a member of the coronavirus group and typically exists as pleomorphic, although generally spherical, virus particles 80 to 120 nm in diameter with coronas of widely spaced club shaped surface projections about 20 nm in length (1, 9). However, the presence or absence of the surface projections varies with different strains (6).

Previous studies on IBV have shown the presence of several virus peaks in sucrose gradients (2, 3) with buoyant densities varying from 1.12 to 1.22 g/cm$^3$, although there usually seems to be a main peak of virus activity with respect to infectivity (4, 10).

It has been observed that IBV particles have 14 to 16 polypeptides (2, 3). Other studies on the polypeptide composition of coronaviruses have shown 6 polypeptides in the virus particles of human coronavirus (HCV) strain OC43 (7)

and transmissible gastroenteritis virus (TGEV) (5), and 7 polypeptides in the virus particles of HCV strain 229 E (8). In the present study we have used less harsh conditions for the analysis of the IBV polypeptides and have obtained fewer polypeptides: this reduces the apparent differences between the polypeptide structure of these morphologically similar viruses.

## Materials and Methods

### *Virus Strains*

IBV strains Beaudette (IBV 42), Connecticut (IBV 46) and Massachusetts (IBV 41) were used. These strains have had a long history of passage in embryonated chicken eggs before their use in the present studies. The Beaudette strain is serologically similar to the Massachusetts strain as it is a Massachusetts strain that has been highly passaged in eggs.

### *Virus Growth*

$10^4$ to $10^5$ $TCD_{50}$ of virus was inoculated by the allantoic route into 10 day old embryonated chicken eggs which were then incubated at 37° C for 24 hours. The allantoic fluid was chilled at 4° C overnight, harvested and immediately clarified by centrifugation at $2000 \times g$ for 30 minutes at 4° C.

### *Virus Purification*

All the purification steps were performed at 0° to 4° C. The virus was pelleted at $75{,}000 \times g$ for 1 hour and then resuspended in 1 ml Dulbecco's phosphate buffered saline 'A' (PBSA). The resuspended virus was overlaid on to a linear 25 to 55 per cent (w/w) sucrose gradient in PBSA and centrifuged for 16 hours at $90{,}000 \times g$. The virus peak(s) were collected, diluted in PBSA and again layered on to linear 25 to 55 per cent (w/w) sucrose gradients in PBSA and centrifuged for 16 hours at $90{,}000 \times g$.

### *Electron Microscopy*

Virus samples were examined after negative staining with 2 per cent (w/v) potassium phosphotungstate, pH 6.5, in a Phillips EM 300 electron microscope.

### *Polyacrylamide Gel Electrophoresis*

Virus peak(s) from the second sucrose gradient were collected and sodium dodecyl sulphate (SDS) and 2-mercaptoethanol were added to concentrations of 5 and 2 per cent respectively and the samples were incubated in a boiling water bath for 1.5 minutes. In some cases the samples were dialysed overnight at room temperature against 5 mM Tris, 38 mM glycine buffer (pH 7.8) containing 1 per cent SDS, 0.1 per cent 2-mercaptoethanol, 3.3 M urea and 5 per cent sucrose (2). Before gel electrophoresis a trace amount of bromophenol blue dye was added and in some cases the virus solution was concentrated using Ultra-Thimbles (Schleicher and Schüll). In a modified procedure of polypeptide preparation the virus solutions were boiled for 3 minutes before overnight dialysis and for 1 minute after dialysis.

Electrophoresis was carried out on cylindrical polyacrylamide gels. The acrylamide concentration was 7.5 per cent (w/v) and the acrylamide: bis-acrylamide ratio was 37.5:1 by weight. The gels also contained 0.1 per cent SDS, 0.5 M urea, 0.03 per cent N,N,N′,N′-tetramethylethylenediamine, 0.07 per cent ammonium persulphate and 0.375 M Tris-HCl buffer, pH 7.8. The gels were pre-electrophoresed at 100 V for 2 hours in an electrophoresis buffer consisting of 25 mM Tris, 192 mM glycine, 0.1 per cent SDS, 0.5 M urea and 0.1 per cent 2-mercaptoethanol, pH 7.8. After pre-electrophoresis, samples were layered directly on top of the gels and electrophoresis was carried out at 100 V until the bromophenol blue dye was approximately 1 cm from the bottom of 10 cm gels.

*Polypeptide Analysis*

After electrophoresis the gels were removed from their supporting tubes and stained for proteins. Staining was with 0.1 per cent Coomassie brilliant blue in 50 per cent methanol, 7.5 per cent acetic acid for 16 hours and destaining was carried out over 2 to 3 days with several changes of 50 per cent methanol, 7.5 per cent acetic acid.

The mobilities of the virus polypeptides were measured relative to the bromophenol blue dye and compared with the mobilities of proteins of known molecular weight that had been reduced, electrophoresed as above and stained with Coomassie brilliant blue. The proteins used as standards were obtained from Sigma Chemical Co. Ltd., and comprised bovine serum albumin (dimer and monomer), ovalbumin, trypsin and lysozyme. The gels were scanned at 620 nm using a Joyce Loebl Chromoscan.

## Results

*Virus Purification*

Centrifugation of IBV particles on sucrose density gradients produced a relatively sharp peak of material ranging in density from 1.17 to 1.18 g/cm$^3$ depending upon the strain of IBV used (Table 1). In 30 per cent of cases an extra peak, forming up to 40 per cent of the total material, was found sedimenting at a density of 1.21 to 1.22 g/cm$^3$. Material also sedimented as a diffuse band in sucrose gradients at a density of 1.13 g/cm$^3$ in 5 per cent of preparations (Table 1). There was no difference in the relative amounts of these peaks over a series of experiments for the different IBV strains. All the virus peaks were infectious, although comparative studies on the infectivity of these peaks were difficult as IBV is extremely labile (3) and large drops in infectivity were observed even after minimal treatment of the virus particles.

Table 1. *Buoyant densities of IBV strains on sucrose gradients*

| IBV strain | Mean density of virus peaks (g/cm$^3$)[a] | | |
|---|---|---|---|
| | Major virus[b] | High density[b] virus | Low density[c] virus |
| Beaudette | 1.18 (1.179—1.184) | 1.22 (1.218—1.223) | 1.13 (1.131—1.136) |
| Connecticut | 1.18 (1.181—1.183) | 1.22 (1.216—1.226) | 1.13 (1.131—1.135) |
| Massachusetts | 1.17 (1.172—1.178) | 1.22 (1.218—1.230) | 1.13 (1.131—1.132) |

[a] The figures in brackets indicate the range of values obtained
[b] Results from 5 experiments
[c] Results from 2 experiments

*Morphology of the Virus Particles*

Figure 1 shows electron micrographs of IBV Beaudette virus particles from the major peak (Fig. 1A) and from the high density peak (Fig. 1B) of sucrose density gradients. There was never enough virus material from the low density peak to study the morphology of these virus particles by electron microscopy.

Essentially, there was little morphological difference between the two forms of the virus particles although the high density form of the virus appeared slightly larger than the major density form. Virus particles from Beaudette were the same size and shape as Connecticut and Massachusetts: all the strains had overall diameters ranging from 80 to 110 nm.

Virus particles in most preparations contained only partial coronas of surface projections and in a few cases almost no surface projections, although in no case did the virus particles contain complete coronas. We only used virus particles that contained partial coronas as they occurred more frequently and appeared more typical of coronaviruses generally. The virus particles with no coronas will be discussed in a later paper.

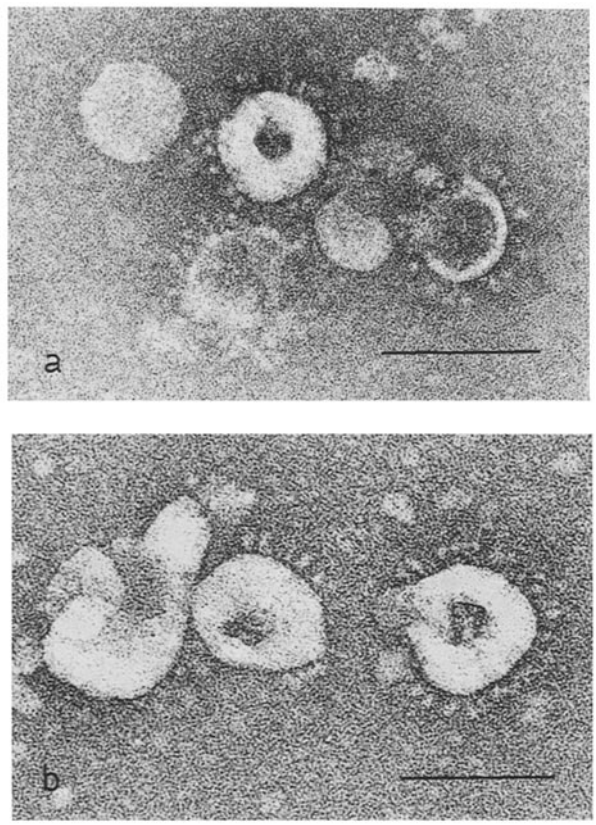


Fig. 1. Particles of IBV Beaudette from sucrose density gradients. a. Major virus (1.18 g/cm$^3$), b. High density virus (1.22 g/cm$^3$). Negative staining with 2 per cent potassium phosphotungstate, pH 6.5. The bar represents 100 nm

*Virus Polypeptide Analysis*

Figure 2 shows the polypeptides of the purified major virus species of the IBV strains Beaudette, Connecticut and Massachusetts, on polyacrylamide gels. Seven polypeptides, VP1 to VP7, were observed in each of the strains. However,

polypeptides VP4 and VP5 could not in all cases be clearly distinguished. The proportions of these polypeptides were similar, apart from the proportion of VP6 which varied considerably in the 3 strains. There was more VP6 in Massachusetts (Fig. 2C) and Connecticut (Fig. 2B) than in Beaudette (Fig. 2A).

Figure 3 shows a comparison between the polypeptides of the major (Fig. 3A) and high density (Fig. 3B) virus species of Massachusetts. The polypeptide profiles were similar except that there was considerably more VP6 in the major species than in the high density species. Similar results have been obtained with Beaudette and Connecticut.

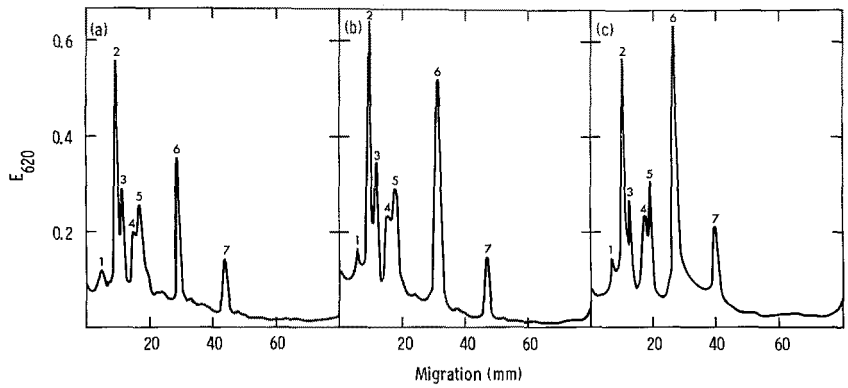


Fig. 2. Densitometer tracings of virus polypeptides on 7.5 per cent polyacrylamide gels after staining with Coomassie brilliant blue. A. Beaudette strain, B. Connecticut strain and C. Massachusetts strain

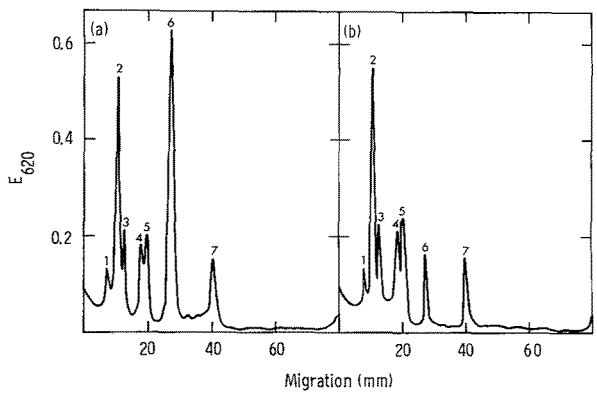


Fig. 3. Densitometer tracings of virus polypeptides on 7.5 per cent polyacrylamide gels after staining with Coomassie brilliant blue. A. Massachusetts major virus species, B. Massachusetts high density virus species

The approximate molecular weights of the virus polypeptides have been determined by comparison with the relative mobilities of bovine serum albumin (dimer and monomer), ovalbumin, trypsin and lysozyme. Table 2 shows the molecular weights of the viral polypeptides determined from 10 experiments.

Table 2. *Molecular weight of the polypeptides of IBV*

| Peptide[b] | Approximate molecular weight[a] $\times 10^{-3}$ | |
|---|---|---|
| | Mean | Range |
| VP1 | 130 | (135—117) |
| VP2 | 105 | (110—100) |
| VP3 | 97 | (99—94) |
| VP4 | 82 | (88—79) |
| VP5 | 74 | (78—70) |
| VP6 | 51 | (53—50) |
| VP7 | 33 | (35—27) |

[a] Results from 10 experiments
[b] VP—Virion Polypeptide

Previous papers on the polypeptide composition of IBV have shown the presence of 14 to 16 polypeptides in the virus particles (2, 3). When we prepared virus polypeptides for electrophoresis using 2 per cent dithiothreitol to reduce the polypeptides (3) or by boiling the polypeptides before and after overnight dialysis (2), we obtained polypeptide patterns similar to those reported previously (2, 3). Figure 4 shows a profile of IBV Beaudette polypeptides prepared using our modified preparative procedure involving boiling of the polypeptides after overnight dialysis (see Methods). Although the pattern varied from experiment to experiment, at least 12 polypeptides were seen compared with the 7 polypeptides observed using our standard procedure (Fig. 2A). The new polypeptides have been designated VPA, VPB, VPC, VPD and VPE and have molecular weights of approximately 56,000, 45,000, 38,000, 21,000 and 19,000 respectively. However, in all cases the proportions of the new polypeptides increased with more degradation of the polypeptides VP1 to VP7. There was essentially no difference between the polypeptide profiles of the major and high density virus species.

Generally, even harsher preparative procedures (such as boiling the samples for 5 minutes before dialysis and 2 minutes after overnight dialysis) removed most

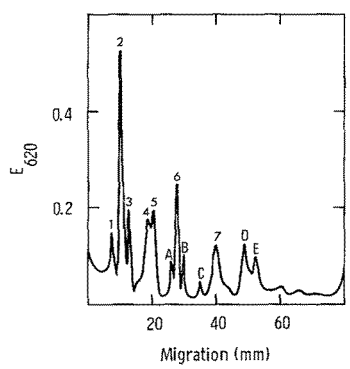


Fig. 4. Densitometer tracing of Beaudette polypeptides on 7.5 per cent polyacrylamide gel after staining with Coomassie brilliant blue. The polypeptides were prepared for gel electrophoresis using the modified procedure described in the Methods

of VP1, VP2 and VP3 and increased the proportions of VPA, VPD and VPE. Similar results were obtained using Connecticut and Massachusetts.

## Discussion

In purified IBV preparations run on sucrose density gradients we saw a major virus peak and up to two others of different densities. The peaks were seen with three different IBV strains and electron microscope studies showed that the viruses from these peaks were morphologically similar and there was little or no contaminating cellular debris.

The polypeptide compositions of the virus particles isolated from the different peaks were similar in that they all contained 7 polypeptides, although there was considerably less VP6 in the high density peak than in the major virus peak. It is interesting to note that the proportion of VP6 also varied considerably between the three IBV strains examined: there was more VP6 in Connecticut and Massachusetts than in Beaudette. As mentioned above, the Beaudette strain is serologically similar to Massachusetts from which it has been derived by multiple passages in embryonated chicken eggs. However, our results suggest that the peptide composition of Beaudette now appears to be more similar to Connecticut than to Massachusetts.

Harsher conditions of polypeptide preparation produced polypeptide profiles containing more than 12 polypeptides, instead of the 7 polypeptides that were usually obtained. Furthermore, considerable variation occurred in the amount, proportion and resolution of these polypeptides with this harsher treatment. From such observations we suggest that these new polypeptides are degradation products and that IBV particles contain only 7 polypeptides.

Six or seven polypeptides have been observed in the virus particles of HCV OC43 (7) and 229E (8) and TGEV (5). A similar number of polypeptides has been identified in mouse hepatitis virus (MACNAUGHTON and MADGE, unpublished results). Thus the polypeptide composition of these coronaviruses resemble that of IBV, although none is identical.

## Acknowledgments

We are indebted to Dr. D. A. J. Tyrrell for his advice and encouragement throughout these studies. We wish to thank Dr. S. Patterson and Mrs. Heather A. Davies for the electron microscopy.

## References

1. BERRY, D. M., ALMEIDA, J. D.: The morphological and biological effects of various antisera on avian infectious bronchitis virus. J. gen. Virol. **3**, 97—102 (1968).
2. BINGHAM, R. W.: The polypeptide composition of avian infectious bronchitis virus. Arch. Virol. **49**, 207—216 (1975).
3. COLLINS, M. S., ALEXANDER, D. J., HARKNESS, J. W.: Heterogeneity of infectious bronchitis virus grown in eggs. Arch. Virol. **50**, 55—72 (1976).
4. CUNNINGHAM, C. H.: Avian infectious bronchitis. Adv. vet. Sci. comp. Med. **14**, 105—148 (1970).
5. GARWES, D. J., POCOCK, D. H.: The polypeptide structure of transmissible gastroenteritis virus. J. gen. Virol. **29**, 25—34 (1975).

6. HARKNESS, J. W., BRACEWELL, C. D.: Morphological variation among avian infectious bronchitis virus strains. Res. vet. Sci. **16**, 128—131 (1974).
7. HIERHOLZER, J. C., PALMER, E. L., WHITFIELD, S. G., KAYE, H. S., DOWDLE, W. R.: Protein composition of coronavirus OC43. Virology **48**, 516—527 (1972).
8. HIERHOLZER, J. C.: Purification and biophysical properties of human coronavirus 229E. Virology **75**, 155—165 (1976).
9. MCINTOSH, K.: Coronaviruses: A comparative review. Curr. Top. Microbiol. Immunol. **63**, 85—129 (1974).
10. TANNOCK, G. A.: The nucleic acid of infectious bronchitis virus. Arch. ges. Virusforschung **43**, 259—271 (1973).

Authors' address: Dr. M. R. MACNAUGHTON, Division of Communicable Diseases, Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ, England.

Received March 30, 1977