The Polypeptides of Infectious Bronchitis Virus (IBV-41 Strain)

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SUMMARY

The Massachusetts strain of avian infectious bronchitis virus was purified from embryonated hens' eggs. Four major species of apparent mol. wt. 90000, 52000, 29000 and 26000 were resolved by SDS-polyacrylamide gel electrophoresis. Omission of reducing agent failed to resolve the 29000 mol. wt. component. Labeling of acrylamide gels with 125I-concanavalin A indicated that polypeptides of mol. wt. 90000, 29000 and 26000 were glycosylated and, in the absence of reducing agent, that the 29000 species migrated as a dimer in the 50000 mol. wt. region. Purified IBV radio-iodinated with Bolton and Hunter reagent, which banded as a single peak of radioactivity in Metrizamide gradients, was found to contain bands of radioactivity when analysed by SDS-PAGE, corresponding to the polypeptides of mol. wt. 90000, 52000 and 29000 resolved in stained gels. Disruption of IBV particles in Triton X-100 released two subviral particles, a 16 nm spike which comprised polypeptides of 90000, 52000 and 29000 mol. wt. and another denser spherical particle of 25 to 45 nm which contained RNA and the 52000 and 26000 polypeptides.

INTRODUCTION

Examination by electron microscopy has shown that avian infectious bronchitis virus (IBV) possesses the distinctive morphology of the family Coronaviridae (Tyrrell et al. 1968). Recent studies have shown that IBV and other coronaviruses possess single stranded RNA genomes of mol. wt. \(6 \times 10^6\) to \(9 \times 10^6\). Other studies have indicated that at least a proportion of virus nucleic acid may be directly translated into virus protein, (Lomniczi & Kennedy, 1977; Schochetman et al. 1977).

The protein structure of several mammalian coronaviruses has been established. Both transmissible gastroenteritis and haemagglutinating encephalomyelitis viruses of swine appear to contain at least two major components with similar mol. wt. (Garwes & Pocock, 1975; Pocock & Garwes, 1977). Of these, one in the mol. wt. range of 50000 to 56000 represented the nucleocapsid protein, a finding similar to that reported for mouse hepatitis virus (Sturman, 1977). The polypeptide profiles of human coronavirus strains OC43 and 229E have also been analysed and found to be similar (Hierholzer et al. 1972; Hierholzer, 1976). In contrast, there has been considerable variation in the reported protein composition of IBV (Bingham, 1975; Collins, et al. 1976; Alexander & Collins, 1977; Macnaughton & Madge, 1977). Several of these workers have shown an extensive heterogeneity of virus-specific material in preparations obtained by density gradient techniques. Virus recovered

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in the buoyant density range 1.12 to 1.22 g/ml has been found to contain from seven to as many as 16 separate polypeptide species as resolved by SDS-PAGE.

In the present study, the optimum conditions for the purification of avian infectious bronchitis virus (IBV-41 strain) were determined. The protein composition was analysed after radio-iodination and the results compared with previously published data.

METHODS

Infectious bronchitis virus (IBV). The Massachusetts strain (M41) of infectious bronchitis virus was used for the present studies. The virus seed was kindly supplied by Dr J. H. Darbyshire of the Houghton Poultry Research Station, Huntingdon, U.K., and on receipt was further passaged three times in embryonated hens' eggs. The allantoic fluid obtained 2 days after infection was used as stock virus for all experiments.

Estimation of virus infectivity. Virus infectivity was quantified by the inoculation of 10-day old embryonated eggs with 0.1 ml of virus diluted in 0.05 M-tris-HCl buffer, pH 7.5. Embryos were examined by dissection 8 days later and virus infection scored by visible evidence of stunting, curling and membrane thickening.

Virus growth and purification. Approx. 100 EID_{50} of virus stock were inoculated into 10-day old embryonated hens' eggs and incubated for 40 h at 37 °C. The eggs were then chilled for a minimum of 4 h at 4 °C and the allantoic fluid harvested and pooled. Virus purification began immediately after a preliminary clarification step; cellular debris was removed by centrifugation for 30 min at 10000 g. Where possible, all steps were performed at 4 °C. Sodium chloride was added to the allantoic fluid with constant stirring to a final concentration of 2.7% and 50%, w/v, solution of polyethylene glycol 6000 (PEG) was then added dropwise until the final concentration reached 8.7%, w/v. The mixture was left for 12 h at 4 °C, centrifuged for 30 min at 10000 g and the pellet resuspended in 0.02 M-tris-HCl buffer, pH 7.0, containing 0.2 M-glycine and 0.002 M-EDTA (GNTE buffer). The final volume corresponded to 1/200th of the original volume of allantoic fluid. Resuspended virus was sonicated in a bath-type sonicator (Headland 3 transducer activated 150 W) for 1 min then centrifuged at 10000 g for 10 min to remove any aggregated material. The concentrated virus suspension was incorporated into the top 5 ml of a 5 to 45%, w/v, Metrizamide (Nyegaard & Co. As. Oslo) gradient and centrifuged at 50000 g in a Beckman SW40 rotor at 4 °C for 8 h. The band containing the peak of infectivity was recovered and separated from Metrizamide by chromatography through a 1.5 x 20 cm column of Sephadex G75 equilibrated in GNTE buffer. The equilibrium centrifugation step was repeated and the purified virus again separated from Metrizamide by chromatography through a 0.9 x 10 cm column of Sephadex G75. Virus was either immediately radiolabelled or stored at -70 °C prior to use.

Radio-iodination of purified IBV. Purified IBV was radiolabelled by the addition of 5 to 10 μg of virus particles diluted in 0.2 M-borate buffer, pH 8.5, to 500 μCi of N-succinimidyl 3-(4-hydroxy, 5-125I-iodophenyl) propionate (Bolton & Hunter, 1973) and left at 4 °C overnight. Unreacted labelled ester was inactivated by the addition of 0.2 M-glycine in 0.2 M-borate buffer and the whole reaction mixture passed through a pre-packed column of Sephadex G25 (Product PD-10, Pharmacia Fine Chemicals, Uppsala, Sweden) equilibrated in PBS + 0.25% gelatin buffer. The radiolabelled virus was further separated by equilibrium centrifugation in a 5 to 45%, w/v, Metrizamide gradient for 4 h at 50000 g. Radiolabelled virus prepared in this manner was stored at 4 °C for up to 1 week, or at -70 °C for 1 month, before further analysis.

SDS-PAGE. Polypeptide analysis of IBV was performed by electrophoretic separation in 6 mm diam. cylindrical polyacrylamide gels using a high pH/discontinuous buffer system (Laemmli, 1970; Maizel, 1971). Gels were poured to a length of approx. 10 cm and overlaid.
IBV polypeptides

with stacking gel to a height of 1 cm. Samples of IBV were solubilized in buffer containing 0.06 M-tris-phosphate, pH 6.7, and 2% SDS. When required, 2-mercaptoethanol was added to a final concentration of 5%, w/v. Disruption was by heating at the temperatures specified in Results below. Separated components were visualized by staining with 0.25% Coomassie brilliant blue R grade, dissolved in fixative solution containing methanol: acetic acid: H2O (64:23:64). After 2 h at room temperature, gels were destained in 5% methanol + 7.5% acetic acid. Reference proteins were run in parallel gels as markers of mol. wt. and included phosphorylase a (mol. wt. 93000), bovine serum albumin (mol. wt. 69000), ovalbumin (mol. wt. 43300), chymotrypsinogen A (mol. wt. 25500) and lysozyme (mol. wt. 14300). Stained gels were scanned in a Joyce-Loebl Chromoscan 200 at 620 nm and the presence of radioactivity determined by counting 1 mm slices in a LKB Ultragamma Model 1280 well-type scintillation counter.

Treatment of IBV with bromelain. Bromelain was obtained in powdered form from Serva Feinbiochemica, Heidelberg. Purified virus was treated in GNTE buffer containing 0.05% and 0.1% bromelain for 1.5 h at 37 °C. Treated virus was then placed directly on to a 10 to 40%, w/v, Metrizamide gradient in GNTE buffer and centrifuged for 10 h at 50000 g in a Sorvall AH-650 rotor. A control preparation was similarly heated in the absence of the enzyme and centrifuged in parallel with treated virus. Bromelain-treated and control viruses were then dialysed overnight against GNTE buffer at 4 °C and then analysed by both electron microscopy and SDS-PAGE.

125I-concanavalin A labelling of IBV polypeptides. Concanavalin A (100 μg; Pharmacia Fine Chemicals) was radiolabelled by the addition of Bolton and Hunter reagent according to the method used for labelling purified IBV (see above) and stored at −70 °C prior to use. Protein components of purified IBV were separated in polyacrylamide gels by electrophoresis, fixed and stained. The gels were washed for at least 2 days in several changes of 0.05 M-tris-HCl buffer, pH 7.2, containing 0.14 M-NaCl and then cut into 1 mm slices. Each slice was immersed in 100 μl of 0.05 M-tris-HCl buffer, pH 7.2, containing 0.14 M-NaCl, 1 mM-CaCl2, 1 mM-MnCl2, 0.5% haemoglobin and 125I-concanavalin A (diluted to approx. 1 x 10^6 ct/min per gel slice). A control gel was similarly treated except that Mn2+ and Ca2+ were not added to the buffer and 5% methyl mannoside (Sigma Chemical Co. St. Louis, Mo., U.S.A.) was included. The slices were incubated in buffer overnight at room temperature and then washed three times in 0.05 M-tris buffer. The presence of residual radioactivity was then determined for each slice.

Triton X-100 disruption of virus. Triton X-100 (Scintillation grade) was obtained from B.D.H. Chemicals Ltd., Poole, Dorset. Purified virus was solubilized in 2% Triton X-100 in GNTE buffer containing 0.5% NaCl for 4 h at 37 °C. Treated virus was then placed directly on to a 20 to 65%, w/v, sucrose gradient in GNTE buffer and centrifuged for 24 h at 150000 g in a Sorvall AH-650 rotor. Protein bands were dialysed against GNTE for 6 h at 4 °C then analysed by electron microscopy and SDS-PAGE. The experiment was repeated using Metrizamide gradients instead of sucrose for separation of components.

Electron microscopy. Samples for electron microscopy were placed on to Formvar/carbon 400 grids, washed with distilled water and stained with 2% phosphotungstic acid adjusted to pH 6.0 by the addition of KOH and examined using an AEI 801 electron microscope at a final magnification of 126000.

RESULTS

Virus purification

The precipitation of virus from infected allantoic fluid was achieved by the addition of PEG 6000 and resulted in a 200-fold concentration of the virus with little or no significant
loss of infectivity. Optimum results were obtained by adding PEG to clarified allantoic fluid immediately after harvesting from infected eggs. Further concentration and purification was then carried out by two successive equilibrium centrifugations in 5 to 45% Metrizamide gradients. The total recoveries of protein and infectivity at each stage are shown in Table 1. Although up to five visible bands were present in the gradients after the first centrifugation step in Metrizamide, infectivity was restricted to a single band with an equivalent buoyant density of 1.14 g/ml. Further equilibrium centrifugation of this material produced a single band of the same buoyant density which contained all recovered virus infectivity. Examination by electron microscopy of the final preparation showed the presence of virus particles with a morphology consistent with that of the coronaviruses (Fig. 1). The majority of the particles had retained the distinctive fringe of outer projections. Although the introduction of the second centrifugation step produced a reduction in the amount of total infectious virus recovered, electron microscopy clearly showed the presence of membranous fragments after only one cycle of centrifugation. During the course of these studies, it was further noticed that the use of centrifugal forces in excess of 100 000 g markedly reduced the number of intact virus particles. This was paralleled by a reduction in the amount of infectious virus recovered.

### Table 1. Purification of IBV

<table>
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<tr>
<th>Protein mg/ml</th>
<th>Infectivity EID&lt;sub&gt;50&lt;/sub&gt;/ml</th>
<th>Volume (ml)</th>
<th>Purification factor</th>
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</thead>
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<tr>
<td>Allantoic fluid</td>
<td>3.0</td>
<td>10&lt;sup&gt;6.0&lt;/sup&gt;</td>
<td>2400</td>
</tr>
<tr>
<td>PEG</td>
<td>2.7</td>
<td>10&lt;sup&gt;6.5&lt;/sup&gt;</td>
<td>12</td>
</tr>
<tr>
<td>MET&lt;sub&gt;1&lt;/sub&gt;</td>
<td>0.3</td>
<td>10&lt;sup&gt;6.5&lt;/sup&gt;</td>
<td>6</td>
</tr>
<tr>
<td>MET&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0.2</td>
<td>10&lt;sup&gt;10.0&lt;/sup&gt;</td>
<td>3</td>
</tr>
</tbody>
</table>

* Purification factor is calculated by estimating total infectivity/mg protein at each stage of purification and dividing by the estimate obtained for allantoic fluid.

![Fig. 1. Electron micrograph of purified IBV, negatively stained with phosphotungstate.](image)
Fig. 2. Sedimentation profile (●—●) of 125I-IBV particles in a Metrizamide gradient after centrifugation for 3 h at 50,000 g in a Beckman SW40 rotor.

Fig. 3. SDS-polyacrylamide disc gel electrophoresis in 10% gels of purified IBV solubilized in 2% SDS at 100 °C for 2 min: (a) in the presence of 5% 2-mercaptoethanol; (b) in the absence of 2-mercaptoethanol. Gels were stained with Coomassie Blue.
Radiolabelling of purified IBV

Virus purified by combined PEG precipitation and equilibrium centrifugation in Metrizamide was radioiodinated as described in Methods. Between 9 and 20% of the radioactivity present in the reaction mixture was routinely introduced into the purified virus particles, giving an approximate sp. act. of 6 μCi/μg protein. Equilibrium centrifugation of the radiolabelled product showed that the radioactivity was present as a single homogeneous band with a buoyant density of 1.12 g/ml (Fig. 2), approx. 0.02 g/ml less than unlabelled virus run under similar conditions.

SDS-PAGE analysis of IBV

A total of three major polypeptide species were resolved by SDS-PAGE analysis of purified virus under reducing conditions (Fig. 3a). After staining with Coomassie brilliant blue, the major components were estimated to possess mol. wt. of 90,000, 52,000 and 29,000 respectively. In addition, two minor components with mol. wt. of 38,000 and 48,000 were consistently present. When 2-mercaptoethanol was omitted from the sample preparation mixture, the peak at 29,000 was reduced (Fig. 3b). Virus radiolabelled by the Bolton and
IBV polypeptides

Fig. 6. Identification of IBV glycoproteins. Profile of radioactivity obtained after incubating 
\( ^{125} \text{I}-\text{concanavalin A} \) with sliced 10\% polyacrylamide gels of purified IBV, which had been solubilized in 2\% SDS: ––O, in the presence of 5\% 2-mercaptoethanol; O––O in the absence of 2-mercaptoethanol.

Hunter method was also analysed by SDS-PAGE. The resulting peaks of radioactivity corresponded to polypeptides with mol. wt. of 90,000, 52,000 and 30,000 with minor peaks of 38,000 and 48,000 thereby indicating that all major polypeptide peaks were radiolabelled (Fig. 4c).

The effect of solubilizing the radiolabelled virus at different temperatures both in the presence and the absence of 2-mercaptoethanol were examined. Solubilization of the virus in the presence of the reducing reagent at 60 °C for 60 min or at 80 °C for 10 min produced little change in either the number or estimated mol. wt. of the resolved polypeptides as compared to heating at 100 °C for 2 min (Fig. 4). However, solubilization at all three temperatures in the absence of 2-mercaptoethanol failed to resolve the 29,000 mol. wt. polypeptide (Fig. 5).

Identification of IBV glycoproteins

Polyacrylamide gels containing reduced or unreduced polypeptide species of solubilized, unlabelled virus were incubated with \( ^{125} \text{I}-\text{concanavalin A} \). After extensive washing, the counting of sliced gels containing reduced protein revealed the reaction of the lectin with two polypeptide species, corresponding to the major components with estimated mol. wt. of 90,000 and 29,000 (Fig. 6). No reaction occurred in the presence of 5\% methyl mannoside suggesting that these reactions were specific for carbohydrate. Components separated in the absence of 2-mercaptoethanol were similarly examined. Addition of \( ^{125} \text{I}-\text{concanavalin A} \) resulted in the resolution of a third glycosylated component with an estimated mol. wt. of 50,000 (Fig. 6). Also present was a peak with an estimated mol. wt. of 26,000 which was not resolved as a separate component on staining of 10\% acrylamide gels.

Effect of bromelain on IBV polypeptides

When virus treated with 0.05\% and 0.1\% bromelain was subjected to equilibrium centrifugation in Metrizamide, a single major band of protein was recovered for both concentrations at a buoyant density of 1.14 g/ml. This was similar to the untreated virus control. Treatment of intact virus particles with 0.05\% bromelain resulted in no apparent change in
Fig. 7. Electron micrograph of purified IBV banded in a Metrizamide gradient after being treated for 1½ h at 37 °C with 0·05 % bromelain.

morphology as visualized by electron microscopy (Fig. 7). However, raising the concentration of bromelain to 0·1 % resulted in the removal of projections (Fig. 8).

Analysis of the protein bands by SDS–PAGE resulted in the appearance of several new species of polypeptides and a corresponding reduction in the amounts of all native virion proteins (Fig. 9a, b). No conclusion could be reached from these results alone as to the position in the virion of any protein component.
IBV polypeptides

Fig. 9. SDS–polyacrylamide gel electrophoresis in 10% gels of virus particles which had been subjected to equilibrium centrifugation in Metrizamide gradients after being heated for 1½ h at 37 °C in: (a) 0.05% bromelain and (b) 0.1% bromelain. Samples for electrophoresis were solubilized in 2% SDS and 5% 2-mercaptoethanol at 100 °C for 2 min. Gels were stained with Coomasie Blue.

**Triton X-100 disruption of virus**

Virus was solubilized in 2% Triton X-100 and its component proteins separated by isopycnic centrifugation in linear sucrose gradients. Two bands containing protein were resolved with buoyant densities of 1.23 and 1.32 g/ml respectively. SDS–PAGE of the lighter band resolved two major glycopeptides of mol. wt. 90,000 and 29,000 together with one major non-glycosylated polypeptide of mol. wt. 52,000. Electron micrographs of this band showed the presence of small particles approx. 16 nm long which were similar to the spikes of the corona on intact virus. Resolution was not fine enough to distinguish differences between particles.

SDS–PAGE of the material which pelleted to the bottom of the gradient resolved a nonglycosylated polypeptide of mol. wt. 52,000 and a glycoprotein of mol. wt. 26,000. The $A_{260}/A_{280}$ absorbance ratio of this protein band was approx. 2, compared with 1 for the band at a density of 1.23 g/ml, suggesting the presence of RNA in the band with the heavier buoyant density. Electron micrographs showed that this material contained amorphous particles.
When the experiment was repeated and isopycnic centrifugation was performed in a linear 10 to 50% Metrizamide gradient two bands of protein were again recovered. The lighter band with a buoyant density of 1.17 g/ml contained particles similar in appearance to the particles banding at a buoyant density of 1.23 g/ml in sucrose (Fig. 10). The denser subviral particles pelleted through a density of 1.32 g/ml in Metrizamide and were visualized as aggregates of spheres 25 to 45 nm in diam. by electron microscopy (Fig. 11). The SDS-PAGE profiles of both types of particles separated in Metrizamide were identical to those of the corresponding particles separated in sucrose.
DISCUSSION

In recent years, several comparative studies have investigated the structure and chemical composition of coronaviruses. Although some variation as to both the number and size of the structural proteins has been reported for coronaviruses of mammals, most studies suggest the presence of four to seven polypeptides (Garwes & Pocock, 1975; Hierholzer, 1976; Sturman, 1977; Wege et al. 1979). In contrast, analyses of infectious bronchitis virus (IBV) polypeptides have been extremely variable. As many as 16 polypeptides were reported by Bingham (1975) whereas Macnaughton & Madge (1977) consistently found only seven polypeptide species. This variation probably reflects the widely different procedures adopted by different laboratories for purification and analysis.

Subsequent to initial studies on the Beaudette strain (IBV-42), several strains of IBV have been analysed and include the Massachusetts (IBV-41) and Connecticut (IBV-46) strains. Although serologically similar to the Massachusetts strain (IBV-41), the Beaudette strain has been subjected to high passage in eggs and has as a result lost its infectivity for chickens (McIntosh, 1974). In addition, there is some evidence that the Beaudette strain contains a corona of fewer projections than the other strains (Harkness & Bracewell, 1974). In the present study, the Massachusetts strain was used throughout as a model of IBV which retains infectivity for chickens.

Bingham (1975) found that IBV recovered from infected allantoic fluid produced at least two bands after equilibrium centrifugation in sucrose gradients; for example, the Massachusetts strain banded at buoyant densities of 1.18 and 1.21 g/ml respectively. Although infectivity was present in both preparations, the lighter virus was associated with a higher RNA: protein ratio. Macnaughton & Madge (1977) have since showed the heavier density preparation contains somewhat less of a 51,000 mol. wt. polypeptide species which is believed to be a major nucleocapsid component (Macnaughton et al. 1977). Collins et al. (1976) separated egg-grown virus into as many as five distinct populations of infectious virus by a combination of continuous and discontinuous sucrose gradient centrifugation steps. Buoyant density values ranged from 1.14 to 1.22 g/ml, although the majority of virus was recovered in the range of 1.19 to 1.22 g/ml. In this and a later study from the same laboratory (Alexander & Collins, 1977), virus was first concentrated from infected allantoic fluid using conditions which would preferentially pellet aggregated material and particles of a larger diameter. In the present study, virus was concentrated effectively from large volumes (2.4 l) of infected allantoic fluid by the addition of polyethylene glycol (PEG). Experiments clearly showed that 100% of virus infectivity was recovered at a final PEG concentration of 8.7% in the presence of 0.5 M-NaCl, Table 1). Wege et al. (1979) have also recently found PEG 600 to be a useful method for initially concentrating the JHM strain of murine coronavirus from infected tissue culture fluid. Alexander & Collins (1977) suggested the amido trizoate Metrizamide to be a useful alternative gradient material for the purification of the Beaudette strain of IBV. Two consecutive shallow gradients were used to separate virus from cellular material. After banding to equilibrium, infectious virus was recovered at a buoyant density of 1.14 g/ml, in agreement with the value reported by Alexander & Collins (1977). Rebanding in a similar gradient resulted in recovery of virus as a single band at the same buoyant density value and electron microscopy clearly demonstrated the presence of particles with a typical coronavirus morphology. Particles were relatively uniform in appearance with diam. in the range of 120 to 160 nm. The majority of particles contained evenly spaced projections, many with a complete corona (see Fig. 1). In addition, there was little evidence of penetration by negative stain, in contrast to the toroid appearance of particles prepared by other workers using purification in sucrose gradients. The lower osmotic pressure associated with the use of Metrizamide may therefore preserve the spherical appearance of
coronavirus particles. The reduced viscosity associated with the use of Metrizamide gradient as compared to the sucrose at the same equivalent concentrations also facilitated the preparation of particles with maximum retention of surface projections. In preliminary experiments, centrifugation procedures employing centrifugal forces of 100,000 \( g \) or more resulted in a marked reduction in the integrity of the outer corona.

In the present study, SDS–PAGE of unlabelled or radiolabelled virus resolved fewer components than reported previously for IBV (Fig. 3, 4). The major components migrated with estimated mol. wt. of 90,000, 52,000 and 29,000 respectively. The binding of \( ^{125} \)-concanavalin A to the 90,000 and 29,000 mol. wt. components indicates the glycoprotein nature of these two species (Fig. 6a). In the absence of 2-mercaptoethanol, a third glycoprotein was detected in the region of the 50,000 mol. wt. polypeptide species and a smaller glycoprotein migrating at a rate corresponding to mol. wt. 26,000. In addition, the major polypeptide peak in the region of the 29,000 mol. wt. was absent in those gels of unlabelled and radiolabelled virus run under non-reducing conditions. One explanation of these results may be the presence of two polypeptides in this mol. wt. range; a 26,000 mol. wt. component which is neither labelled by the Bolton and Hunter reagent nor visualized in gels stained with Coomassie blue, possibly owing to a high carbohydrate content. Alternatively, there may be only one polypeptide of 29,000 mol. wt. which, in the absence of 2-mercaptoethanol migrates mainly as a dimer with an estimated mol. wt. of 50,000 but also exists as a monomer migrating slightly faster at 26,000 mol. wt. compared with an estimated mol. wt. of 29,000 after reduction with 2-mercaptoethanol.

In order to study further the structure of IBV, virions were disrupted in 2% Triton X-100 resulting in the production of two types of subviral particles. The smaller was approx. 16 nm long and had a buoyant density of 1.23 g/ml in sucrose and probably comprised the spikes of the corona. The larger particle was spherical in shape, 25 to 35 nm in diam., possessed a buoyant density in excess of 1.32 g/ml and was probably the core containing RNA. SDS–PAGE analysis of each type of particle indicated that two glycopeptides of 29,000 and 90,000 mol. wt. and a non-glycosylated polypeptide of 52,000 were present in the spikes and a non-glycosylated 52,000 mol. wt. species and a glycopeptide of 26,000 mol. wt. comprised the core. The observation that the denser subviral particle retained its spherical shape in Metrizamide but not sucrose probably reflects the difference in osmolarity between the two gradient materials and may explain why earlier attempts to define the internal structure of IBV were not successful.

The results obtained when IBV was disrupted with non-ionic detergents are similar to those reported for HEV (Pocock & Garwes, 1977), TGE (Garwes et al. 1976), and MHV (Wege et al. 1979). In all studies, a fast sedimenting spherical particle was released which contained a non-glycosylated polypeptide of approx. 50,000 mol. wt. and had associated with it at least one small mol. wt. glycopeptide; 26,500 in HEV, 24,800 in MHV and 30,000 and 28,500 in TGE. A second non-glycosylated polypeptide was also found to be present in the spherical subviral particle in MHV. The reported size of the subviral particle varied among these three coronaviruses.

These results suggest the existence in IBV of two glycoproteins in the lower mol. wt. range. One, of 29,000 mol. wt., is linked to itself or another polypeptide by disulphide bridges to form a larger peptide and exists on the surface of the virion where it can be radio-labelled by the Bolton and Hunter reagent. The other has a mol. wt. of 26,000 and is not labelled by the Bolton and Hunter reagent possibly because it has no free amino groups external to the lipid bilayer.

The presence in the virion of two non-glycosylated polypeptides migrating in the 52,000 mol. wt. range may help to explain why bromelain reduces the amount of this polypeptide, the component external to the lipid bilayer being affected but not the internal polypeptide.
IBV polypeptides

Alternatively there may be only one 52,000 mol. wt. polypeptide some of which migrates with the peplomers and the rest with the ‘core’ when the virus structure is disrupted by Triton X-100. This implies that bromelain can penetrate the envelope.

Other studies have indicated the presence in many coronaviruses of at least one polypeptide in the size range of 180,000 to 200,000. However, Sturman (1977) has suggested that the 180,000 mol. wt. component of mouse hepatitis virus (MHV) contains amino acid sequences common to a smaller 90,000 mol. wt. component. This latter polypeptide may therefore be a cleavage product of a higher mol. wt. component. Further studies are required in order to define precisely the relationship of these components and the intracellular events accompanying coronavirus maturation.

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