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Structural Polypeptides of Coronavirus IBV

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SUMMARY

Avian infectious bronchitis virus (IBV) was grown and radiolabelled with ^{35}S-methionine, ^{3}H-leucine and ^{3}H-glucosamine in de-embryonated chicken eggs. Approximately 12 different polypeptides were clearly detected by SDS–polyacrylamide gel electrophoresis of virus preparations. Growth of IBV in chorioallantoic membrane cells labelled with ^{35}S-methionine indicated that most of these polypeptides, and additional ones, some of which were glycosylated, were host components. Five polypeptides appeared to be virus-coded, with apparent mol. wt. of 94×10^3, 84×10^3, 54×10^3, 30×10^3 and 28×10^3. Four of these, p94, p84, p30 and p28, were glycosylated. The virion spikes appeared to be composed of p94 and p84, while p30 and p28 were partially embedded in the virion membrane. By analogy with other reports, p54 is the nucleocapsid polypeptide.

INTRODUCTION

Avian infectious bronchitis virus (IBV) is a member of the family *Coronaviridae*, of which there is one genus, *Coronavirus* (Tyrrell *et al.*, 1978). The genome of IBV is a single-stranded RNA of mol. wt. 6×10^6 to 9×10^6 (see Tyrrell *et al.*, 1978 for references) and is surrounded by a lipid envelope with which are associated the large, club-shaped surface projections (spikes) characteristic of the coronaviruses.

The polypeptide composition of several mammalian coronaviruses has been determined, namely human coronavirus (HCV) strains 229E (Hierholzer, 1976; Macnaughton, 1980) and OC43 (Hierholzer *et al.*, 1972), transmissible gastroenteritis virus (TGEV) of pigs (Garwes & Pocock, 1975; Garwes *et al.*, 1976), haemagglutinating encephalomyelitis virus (HEV) of pigs (Pocock & Garwes, 1977), mouse hepatitis virus (MHV) strains A59 (Sturman, 1977; Bond *et al.*, 1979), JHM (Wege *et al.*, 1979; Bond *et al.*, 1979) and MHV3 (Macnaughton, 1980). In general, the polypeptides of these coronaviruses can be assigned to four size-classes (Sturman, 1977). Mol. wt. ranges of 130×10^3 to 200×10^3 and 89×10^3 to 125×10^3 characterize polypeptides in size-classes one and two respectively. Polypeptides of both these classes are glycosylated and constitute the virion spikes (S) (Bingham, 1975; Garwes & Pocock, 1975; Macnaughton *et al.*, 1977; Pocock & Garwes, 1977; Sturman, 1977; Wege *et al.*, 1979; Macnaughton, 1980; Collins & Alexander, 1980*b*). Non-glycosylated polypeptides of 50×10^3 to 60×10^3 mol. wt. comprise size-class three and form the nucleocapsid (N) protein (Garwes *et al.*, 1976; Sturman, 1977; Macnaughton *et al.*, 1977; Macnaughton & Davies, 1980). Size-class four comprises polypeptides of mol. wt. 18×10^3 to 30×10^3. Some reports indicate the presence of only one, glycosylated, polypeptide in this class while others show two polypeptides, at least one of which is glycosylated. Evidence has been presented which suggests that the 23×10^3 mol. wt. glycopeptide of MHV-A59 is largely embedded within the virus membrane (M), and protrudes at both the outer (Sturman, 1977; Sturman & Holmes, 1977; Wege *et al.*, 1979) and inner (Sturman *et al.*, 1980) membrane surfaces.

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In contrast to the mammalian coronaviruses as many as 30 polypeptides have been reported in preparations of IBV (Collins & Alexander, 1980*a*). Many of these are presumably host-coded polypeptides, but no attempt has been made to differentiate virus- and host-coded components, principally because most studies of IBV polypeptides have been made using non-labelled virus grown in embryonated chicken eggs (Bingham, 1975; Collins *et al.*, 1976; Alexander & Collins, 1977; Macnaughton & Madge, 1977; Nagy & Lomniczi, 1979; Collins & Alexander, 1980*a*) although Lanser & Howard (1980) have iodinated IBV after purification. To investigate this problem, and to produce IBV rapidly and economically, I have radiolabelled the polypeptides of IBV during multiplication in de-embryonated chicken eggs. This technique has been used to produce ^{3}H-uridine-labelled IBV (Schochetman *et al.*, 1977) and ^{35}S-methionine-labelled influenza virus (Dimmock *et al.*, 1977). The specific infectivity of the influenza virus from de-embryonated eggs was more than 1000-fold greater than virus labelled in embryonated eggs.

METHODS

Viruses. The Massachusetts M41 strain of IBV was used throughout this work. This had been passaged at limiting dilution in chick embryo trachea organ cultures (Darbyshire *et al.*, 1979). Working stocks of virus were prepared in 9-day-old embryonated chicken eggs which originated from a specific pathogen-free flock. The eggs were inoculated with approx. 100 median ciliostatic dose (CD_{50}) of M41 (Darbyshire *et al.*, 1979). Allantoic fluid was harvested after 45 h at 37 °C and stored at −70 °C.

Influenza virus strain A/Okuda/57 (H_2N_2) was obtained from Dr C. Sweet, University of Birmingham, U.K. The virus was passaged once in 11-day-old embryonated chicken eggs. The allantoic fluid, harvested at 22 h p.i., had a titre of 10·3 $\log_{10}$ egg lethal $dose_{50}$/ml and was stored at −70 °C.

Preparation of radiolabelled virus in de-embryonated eggs. De-embryonated eggs were prepared by the method of Dimmock *et al.* (1977). They were inoculated with 5 ml virus suspension (to give a multiplicity of approx. 7 CD_{50}/surface chorioallantoic membrane (CAM) cell (Cuadra, 1975); this was infectious allantoic fluid which had been clarified and dialysed against 50 vol. of F-medium overnight at 4 °C. F-medium (Fazekas de St. Groth & White, 1958) comprised (×10 working strength): 80 g/l NaCl; 6 g/l KCl; 8 g/l $CaCl_2.2H_2O$; 0·5 g/l $MgCl_2.6H_2O$; 3 g/l glucose; 10 g/l gelatin (Difco); 0·0025% phenol red; the solution was adjusted to pH 7 with NaOH and was sterilized at 115 °C for 15 min. The titre of the virus preparation was approx. 7·5 $\log_{10}$ CD_{50}/ml and did not decrease during dialysis. Penicillin (300 units/ml) and streptomycin (300 μg/ml) were added to the inoculum. The de-embryonated eggs were then incubated as described by Dimmock *et al.* (1977). After 2·5 h, 250 μl of medium containing radioisotope was added to each egg and incubation was continued for a further 20 h. Three de-embryonated eggs were used for a virus preparation, each egg receiving 80 μCi ^{35}S-methionine (sp. act. 600 Ci/mmol), 110 μCi ^{3}H-leucine (sp. act. 100 to 150 Ci/mmol) or 330 μCi D-6-^{3}H-glucosamine hydrochloride (sp. act. 20 to 40 μCi/mmol), obtained from The Radiochemical Centre, Amersham.

Radiolabelled influenza virus was prepared by inoculation of each of three de-embryonated eggs with 5 ml F-medium, 0·1 ml virus preparation and 80 μCi ^{35}S-methionine or ^{3}H-leucine. Incubation was at 37 °C for 22 h.

Purification of radiolabelled virus. All procedures were performed at 0 to 4 °C. The medium from de-embryonated eggs was clarified at 2500 ***g*** for 5 min. An equal vol. of saturated ammonium sulphate solution pH 6·5 was then added and the mixture stirred for 30 min. The precipitate was recovered by centrifugation at 2500 ***g*** for 15 min and dissolved in 2 ml PBSA. After clarification the virus preparation was layered on to a 25 to 45% (w/w)

sucrose gradient in PBSA containing 20 μg/ml bovine serum albumin (BSA) to minimize loss of virus on tube walls and centrifuged at 30000 ***g*** (average) in an MSE 3 × 25 ml swing-out rotor for 3 h at 4 °C. Fractions were collected by upward displacement and 20 μl samples estimated for radioactivity in a liquid scintillation counter. Selected fractions were pooled, diluted, layered on to a 25 to 55% (w/w) linear sucrose gradient containing 20 μg/ml BSA and centrifuged at 30000 ***g*** (average) in an MSE 6 × 38 ml swing-out rotor for 16 h at 4 °C. The refractive index of each fraction was measured using an Abbé refractometer.

The virus in some harvests was concentrated by pelleting at 30000 ***g*** (maximum) for 3 h or 70000 ***g*** (maximum) for 1·5 h and the pellet resuspended by means of an MSE 150 W ultrasonic disintegrator at maximum amplitude with a 3 mm diam. probe.

Influenza virus from de-embryonated eggs was purified essentially as described by Dimmock *et al.* (1977).

SDS–polyacrylamide gel electrophoresis. Virus polypeptides were analysed by SDS–polyacrylamide gel electrophoresis using the buffer system of Laemmli (1970) and 4·5% (w/v) acrylamide stacking gels in either tubes (10% acrylamide separation gel) or slabs [10 to 22·5% acrylamide separation gel stabilized with a 0 to 50% (v/v) glycerol gradient]. For tube gels virus samples were taken directly from sucrose gradients and denatured in boiling water for 2 min in the presence of 2% (w/v) SDS and 2% (v/v) 2-mercaptoethanol. Denatured marker polypeptides (Sigma) were added to the samples. Their mol. wt., in parentheses, were taken from the list of Weber & Osborn (1969); phosphorylase-a (rabbit muscle: 94000); catalase (bovine liver: 60000); fumarase (porcine heart: 49000); glyceraldehyde-3-phosphate dehydrogenase (yeast: 36000); chymotrypsinogen-A (bovine pancreas: 25700); and lysozyme (egg white: 14300). The samples also contained BSA (68000; Armour pharmaceuticals, Eastbourne, Sussex, U.K.). After electrophoresis the gels were stained with 0·25% Coomassie blue in water : methanol : acetic acid (51 : 40 : 9), destained, cut transversely into 1 mm slices, digested for 2 h at 48 °C in 0·7 ml NCS tissue solubilizer : water (9 : 1; Amersham Corporation, Arlington Heights, Ill., U.S.A.) and radioactivity determined in a scintillation counter.

For slab gels, virus samples were dialysed against water, lyophilized and resuspended in sample buffer (Laemmli, 1970). In addition to the marker polypeptides stated above, the following ^{14}C-methylated polypeptides (The Radiochemical Centre, Amersham) were used (mol. wt. in parentheses): myosin (200000); phosphorylase-b (92500); BSA (68000); ovalbumin (46000); carbonic anhydrase (30000); and lysozyme (14300). After electrophoresis the gels were stained and fluorographs prepared as described by Bonner & Laskey (1974).

Growth of virus in prelabelled cells. Three de-embryonated eggs were incubated at 37 °C with 5 ml F-medium and 100 μCi/egg ^{35}S-methionine. After 6 h, 0·5 ml 10 mM unlabelled methionine was added to each egg and incubation continued for 30 min. Holes were then cut in the foil caps and the medium decanted. The CAMs were washed three times with F-medium and then inoculated with 5 ml of virus with 100 μCi/egg ^{3}H-leucine and 2 mM unlabelled methionine. After a further 22 h incubation at 37 °C virus was precipitated with ammonium sulphate as described above and centrifuged to equilibrium in a linear 25 to 55% (w/w) sucrose gradient.

Treatment of IBV with bromelain, Tween 80 and urea. ^{35}S-methionine-labelled virus, which had been purified by ammonium sulphate precipitation and isopycnic centrifugation, was used. For enzyme treatment, virus was incubated at 37 °C for 90 min with 2 mg/ml bromelain (Boehringer-Mannheim) and 1 mM-2-mercaptoethanol in PBS. Virus preparations were treated with urea by addition of an equal volume of 10 M-urea for 45 min at room temperature. For other virus preparations, Tween 80 [polyoxyethylene (20) sorbitan mono-oleate; BDH] was added to a final concentration of 30 mg/ml immediately before

centrifugation. Virus samples were then centrifuged in 25 to 55% (w/w) linear sucrose gradients at 30000 ***g*** (average) for 16 h at 4 °C.

RESULTS

Sedimentation and specific-infectivity characteristics of IBV

Virus from de-embryonated eggs, which was concentrated by pelleting and sedimented to equilibrium in sucrose gradients, had a mean density of 1·16 g/ml. When virus was concentrated by precipitation with ammonium sulphate the mean density of the virus in sucrose gradients was 1·175 g/ml with smaller quantities of virus in the density range 1·15 to 1·16 g/ml.

Analysis of the specific infectivity of virus particles of different densities from four preparations showed that virus of density 1·17 to 1·18 g/ml had a specific infectivity up to 10-fold greater than particles of density 1·15 to 1·16 g/ml. As shown below, virus of density 1·15 to 1·16 g/ml had only 20 to 30% of the amount of the spike polypeptides in 1·17 to 1·18 g/ml virus. These results indicated that pelleting of IBV produced in de-embryonated eggs generated more low-density particles than did concentration by precipitation. Consequently, virus concentrated by precipitation was used in subsequent studies.

Polypeptides of IBV

Approximately 12 polypeptides were discernible when ^{35}S-methionine-labelled IBV was analysed by SDS–polyacrylamide gel electrophoresis (Fig. 1 *a*). A similar result was obtained with ^{3}H-leucine-labelled virus. Four polypeptides of apparent mol. wt. 94×10^3, 84×10^3, 54×10^3 and 30×10^3 in 10% acrylamide gels accounted for >70% of the ^{35}S-methionine radiolabel present in the gels. The curve corresponding to the 30×10^3 mol. wt. polypeptide was broad, which indicated that two polypeptides of similar mol. wt. might be present (Fig. 1 *a*). In slab gels there was a major band at 30×10^3 mol. wt. and a lesser band at 28×10^3 mol. wt. (Fig. 4). The five polypeptides of mol. wt. 94×10^3, 84×10^3, 54×10^3, 30×10^3 and 28×10^3 correspond well to size classes 2, 3 and 4 of mammalian coronaviruses (see Introduction). However, the total number of polypeptides present was two- to threefold greater than has been reported for other coronaviruses. Attempts were made, therefore, to determine whether the additional polypeptides were virus- or host-coded.

Effect of various conditions on the polypeptide composition of IBV

When IBV polypeptides were electrophoresed after denaturation in 2% SDS ± 2% 2-mercaptoethanol at 37 °C the subsequent polypeptide profile was essentially the same as that obtained from virus denatured at 100 °C for 2 min in 2% SDS and 2% 2-mercaptoethanol. Heating at 100 °C for 5 min followed by dialysis and additional heating at 100 °C did not alter the profile. Thus, the minor polypeptides did not appear to be anomalously migrating virus-coded polypeptides as have been described for mouse coronavirus (MCV; Sturman, 1977; Wege *et al.*, 1979).

The proportion of radiolabel accounted for by the 21×10^3 and 14×10^3 mol. wt. polypeptides varied greatly among different IBV preparations while the ratio of counts in the other polypeptides with respect to those in p54 was almost constant, except for p94 and p84 which varied with the density of the virus (Fig. 1 *a*, *b*). Preparations of influenza virus and some marker polypeptides also contained material of 14×10^3 mol. wt. It is concluded that the material at the 14×10^3 and 21×10^3 mol. wt. positions probably represents degraded polypeptides or contaminants, or both.

Various combinations of rate-zonal and isopycnic conditions did not change either the number or ratio of the radiolabelled polypeptides of IBV. Virus that had been concentrated by

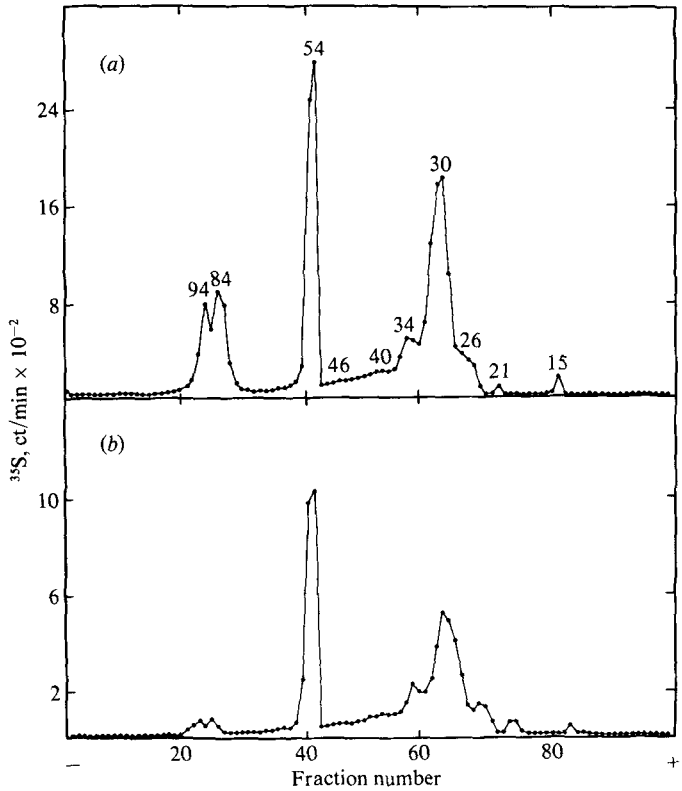

Fig. 1. SDS–polyacrylamide gel electrophoresis of ^{35}S-methionine-labelled IBV of density (*a*) 1·182 g/ml and (*b*) 1·152 g/ml purified by rate-zonal and isopycnic centrifugation. The numbers in this and subsequent figures indicate the apparent mol. wt. × 10^{-3} of the polypeptides.

ultracentrifugation, and which had a mean density of 1·16 g/ml, had a very similar polypeptide composition to that shown in Fig. 1 (*b*) for the low-density virus recovered by ammonium sulphate precipitation, i.e. a small complement of p84 and p94. Addition of 30 mg/ml Tween 80 or 5 M-urea to virus before sedimentation in sucrose gradients removed only one minor polypeptide, that of mol. wt. 75 × 10^3; the amount of label in this polypeptide varied between preparations and was always very low. These findings indicate that most of the ^{35}S-methionine-labelled minor polypeptides in virus preparations were strongly associated with the virus particles.

Presence of host-coded polypeptides in virus preparations

Since up to 30 polypeptides have been observed in unlabelled preparations of IBV from embryonated eggs (Collins & Alexander, 1980*a*), it seemed probable that many of them were of host origin. Attempts were therefore made to determine whether the minor labelled polypeptides associated with IBV from de-embryonated eggs were virus- or host-coded. The approach adopted was to grow virus in the presence of unlabelled methionine, in CAM cells which had previously been labelled with ^{35}S-methionine: cellular polypeptides would then be labelled in preference to virus polypeptides. The ratio of ^{35}S-methionine counts in p54 to those in the normally minor polypeptides might then be expected to be lower than in virus which had been labelled under standard conditions i.e. ^{35}S-methionine added 2·5 h after infection.

^{3}H-leucine was inoculated together with the virus in order to obtain ^{3}H-leucine-labelled virus polypeptides to aid the identification of the ^{35}S-methionine-labelled polypeptides of 94 ×

Table 1. *Presence of host-coded polypeptides in IBV*

Virus	Total ^{35}S (ct/min in gel)*	% ^{35}S (ct/min in p94, p84, p54, p30 and p28)	Ratio ct/min of p54/other polypeptides						
			p30 + p28	p94 + p84	p26	p34	p39	p46	p150
Labelled under standard conditions†	23897	70	0·8	1·4	4·8	3·7	6·7	13·7	140
From prelabelled‡ cells (expt. 1)	11367	23	1·0	1·1	3·3	1·5	1·9	0·9	8
From prelabelled‡ cells (expt. 2)	1587	35	0·8	1·7	2·2	2·4	3·3	1·7	12

* Polypeptides were analysed in 10% polyacrylamide tube gels.

† ^{35}S-methionine was added to the de-embryonated eggs 2·5 h after infection.

‡ De-embryonated eggs were incubated for 6 h with ^{35}S-methionine followed by 30 min with 10 mM unlabelled methionine. The CAM was then washed and inoculated with virus in the presence of 2 mM unlabelled methionine. After 22 h at 37 °C released virus was purified and analysed by SDS–polyacrylamide gel electrophoresis.

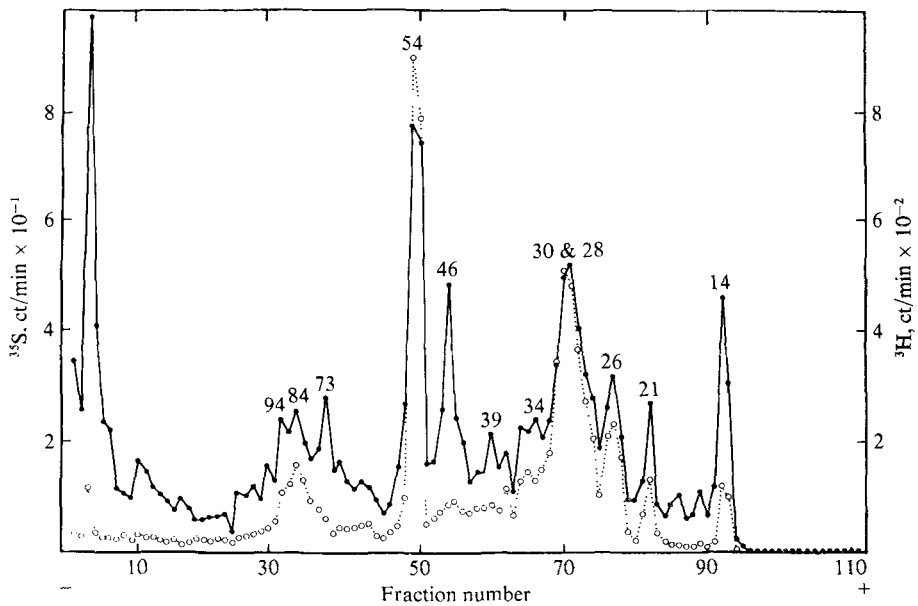

Fig. 2. Presence of host-coded polypeptides in IBV. De-embryonated eggs were prelabelled with ^{35}S-methionine and then inoculated with virus plus ^{3}H-leucine in the presence of excess non-labelled methionine. After 24 h at 37 °C the virus was purified by isopycnic centrifugation and the polypeptides analysed by SDS–polyacrylamide gel electrophoresis: ●——●, ^{35}S; O---O, ^{3}H. The virus analysed in this gel corresponds to expt. 2 in Table 1.

10^3, 84 × 10^3, 54 × 10^3, 30 × 10^3 and 28 × 10^3 mol. wt. In two such experiments p94, p84, p54, p30 and p28 accounted for 23% in one experiment and 35% in the other, of the ^{35}S-methionine counts in gels (Table 1), whereas for virus labelled under standard conditions these polypeptides accounted for >70% of the radiolabel. The ct/min ratio of p54 to p94 + p84 and p30 + p28 for virus from prelabelled cells was similar to that of virus labelled under standard conditions, while the corresponding ratios for the minor polypeptides had decreased by 2- to 14-fold (Fig. 2, Table 1). Several other ^{35}S-methionine polypeptides e.g. >100 000 mol. wt., which were not readily detected in standard virus preparations, were clearly present in virus from prelabelled cells (Fig. 2). These results are consistent with the proposition that

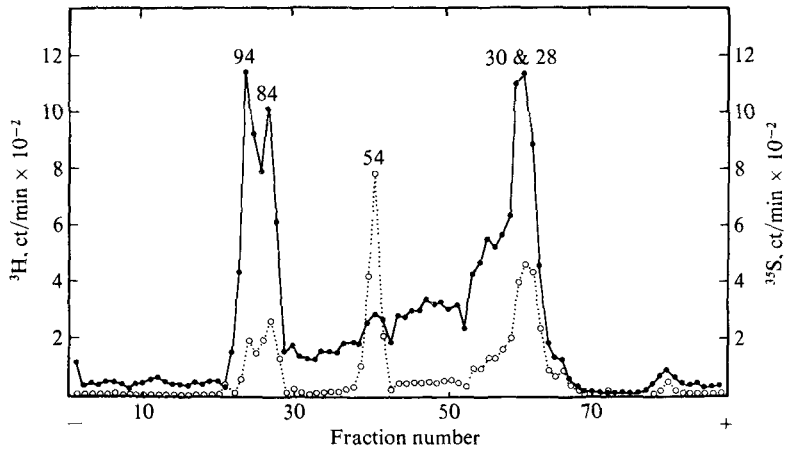

Fig. 3. SDS–polyacrylamide gel electrophoresis of IBV grown in the presence of ^{3}H-glucosamine (●——●) and ^{35}S-methionine (O---O).

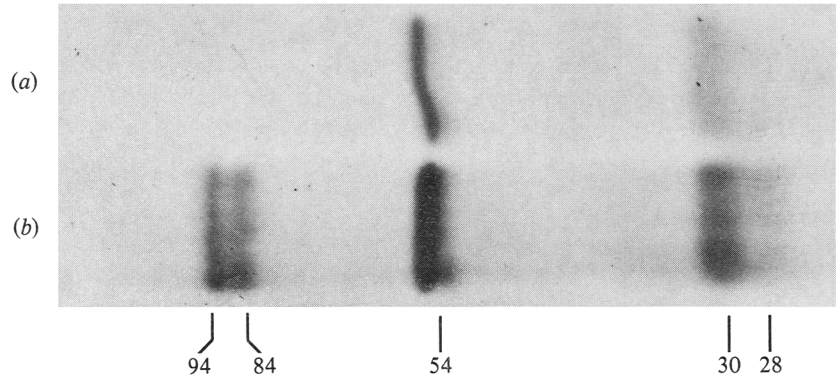

Fig. 4. SDS–polyacrylamide gel electrophoresis of (*a*) 5 M-urea-treated and (*b*) control IBV. The polypeptides were analysed in gradient slab gels and fluorographs subsequently prepared.

most of the polypeptides in IBV preparations, with the exception of p94, p84, p54, p30 and probably p28, are host-coded.

Glycopeptides of IBV

Virus grown in the presence of ^{3}H-glucosamine had label in many polypeptides, including four (p94, p84, p30 and p28, subsequently referred to as gp94, gp84, gp30 and gp28 respectively) of the five virus-coded polypeptides (Fig. 3). The small amount of ^{3}H-glucosamine associated with p54 is probably due to a host-cell polypeptide. Whereas the ratio of ct/min in gp84 to those in gp94 for ^{35}S-methionine- and ^{3}H-leucine-labelled polypeptides was approx. 2, the ct/min ratio of gp84 to gp94 for ^{3}H-glucosamine-labelled material was approx. 1. Assuming that the amount of ^{35}S-methionine and ^{3}H-leucine present is proportional to the quantity of polypeptide, this indicates that gp94 has twofold more glucosamine residues than gp84.

Identification of the spike polypeptides

IBV treated with 5 M-urea had a buoyant density of approx. 0·02 g/ml less than control virus, a 1000-fold lower specific infectivity and no spikes as evidenced by electron microscopy. Electrophoresis of the polypeptides followed by fluorography indicated that gp94 and gp84 were absent (Fig. 4), although analysis of sliced gels showed that a small amount of

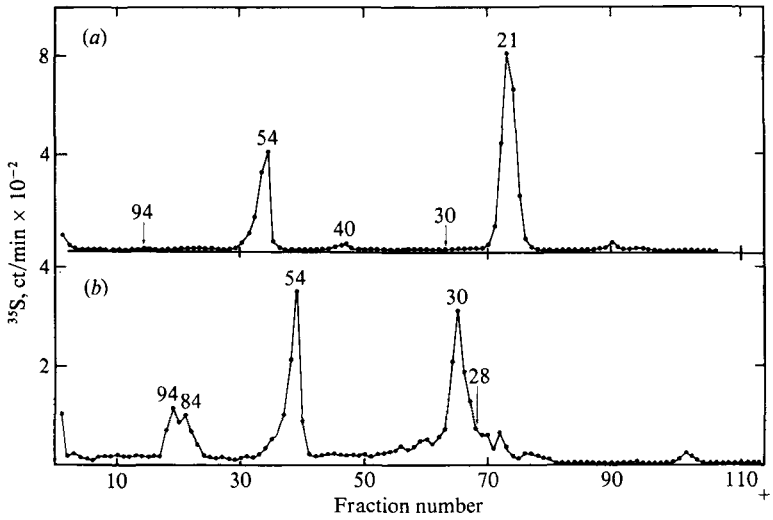

Fig. 5. SDS–polyacrylamide gel electrophoresis of (*a*) bromelain-treated and (*b*) control IBV.

gp84 was still present. This indicates that the spikes (S) were composed of gp94 and gp84. Further evidence for the involvement of both gp94 and gp84 in the composition of the spikes is provided by the observation that virus of low density (1·15 to 1·16 g/ml) had decreased amounts of both these glycopeptides (Fig. 1 *b*) as compared with virus of density 1·17 to 1·18 g/ml (Fig. 1 *a*).

Membrane location of gp30 and gp28

Bromelain has been shown to completely remove the spike polypeptides of IBV and several other coronaviruses (Hierholzer *et al.*, 1972; Garwes & Pocock, 1975; Hierholzer, 1976; Macnaughton *et al.*, 1977; Sturman, 1977) and to partially hydrolyse the size-class four glycopeptide of MCV (Sturman, 1977; Wege *et al.*, 1979). Bromelain-treated IBV had only one virus-coded polypeptide, p54, of unchanged mol. wt., most of the minor polypeptides had also been hydrolysed, and a large peak of 21000 mol. wt., had appeared (Fig. 5). The amount of label in the p21 peak of enzyme-treated virus was more than could be accounted for by any cleavage products of the S polypeptides or the minor polypeptides and was most probably derived from gp30 and gp28. The failure of bromelain to hydrolyse all the mass of gp30 and gp28 suggests that much of these molecules was embedded in the virus membrane and can thus be considered as membrane polypeptides (M1 and M2 respectively). The ct/min ratio of p21/p54 for the bromelain-treated virus (Fig. 5 *a*) was almost twice that of the control virus (Fig. 5 *b*). This indicates that some p54 had been lost and/or hydrolysed by bromelain. Electron microscopy showed that some of the bromelain-treated particles, which lacked spikes, had damaged membranes; this would have made some p54, which by analogy with other reports, is the nucleocapsid (N) polypeptide, accessible to the enzyme. The density and specific infectivity of the bromelain-treated virus was approx. 0·02 g/ml and 100-fold less respectively than for control virus.

DISCUSSION

IBV has been grown and radiolabelled in the CAM of de-embryonated chicken eggs. The virus was heterogeneous with respect to sedimentation rate and buoyant density when isolated by sucrose density-gradient centrifugation, as reported previously for IBV grown in embryonated eggs (Bingham, 1975; Macnaughton & Madge, 1977).

At least 12 polypeptides were distinguished in sliced gels following SDS–PAGE of

^{35}S-methionine-labelled (Fig. 1) and ^{3}H-leucine-labelled IBV (Fig. 2). Of these, five were virus-coded while the remainder were shown to be host-coded (Fig. 2, Table 1). Additional host-coded polypeptides, poorly labelled in standard virus preparations, were also present (Fig. 2). This suggests that the majority of the polypeptides detected in preparations of unlabelled virus from embryonated eggs are host-coded. Experiments with influenza virus, urea, the surface-active agent Tween 80 and various combinations of centrifugation conditions, indicated that most of the minor, host-coded polypeptides were closely associated with the virus. The finding that many of the minor polypeptides were glycosylated (Fig. 3) indicates that they have a membrane origin and that they may have been present in the membranes of the endoplasmic reticulum and Golgi apparatus into which the virus nucleocapsid buds during virus maturation (McIntosh, 1974).

The virion spikes appeared to be composed of gp94 (S1) and gp84 (S2) and p54, by analogy with other reports and its relative insensitivity to bromelain (Fig. 5), was the N polypeptide. Bromelain cleaved gp30 (M1) and gp28 (M2) to a polypeptide of 21 $\times$ 10^3 mol. wt., indicating that approx. 60% of the mass of the M polypeptides was embedded in the virion membrane and was thus inaccessible to bromelain. Macnaughton *et al.* (1977) reported that the 33000 mol. wt. polypeptide of IBV strain Connecticut was not affected by bromelain although these authors also reported that this polypeptide was not glycosylated, contrary to the finding for the size-class four polypeptide of IBV strain M41 (Fig. 3; and Lanser & Howard, 1980) and of IBV strain Connecticut (Collins & Alexander, 1980*a*). Sturman (1977) showed that bromelain cleaved gp23 of MCV to an 18 $\times$ 10^3 mol. wt. polypeptide and made the suggestion, supported by Wege *et al.* (1979), that part of the size-class four glycopeptide of MCV is embedded in the virion membrane.

Comparisons of previous reports on the polypeptide composition of IBV strain M41 have been difficult, partly because of the failure to discriminate between virus- and host-coded polypeptides. However, the N polypeptide of the present communication clearly corresponds to the 51 $\times$ 10^3, 50 $\times$ 10^3, 54 $\times$ 10^3 and 52 $\times$ 10^3 mol. wt. polypeptides of Macnaughton *et al.* (1977), Nagy & Lomniczi (1979), Collins & Alexander (1980*a*) and Lanser & Howard (1980) respectively, while M1 and M2 are probably analogous to the 33 $\times$ 10^3, 28 $\times$ 10^3, 31 $\times$ 10^3 and 28 $\times$ 10^3, and 29 $\times$ 10^3 and 26 $\times$ 10^3 polypeptides of the same authors. B. Lomniczi & J. Morser (personal communication) have shown that the Beaudette strain of IBV, of the Massachusetts serotype, radiolabelled in chicken embryo kidney (CEK) cells, has a major and minor glycopeptide of 30 $\times$ 10^3 and 28 $\times$ 10^3 mol. wt. respectively. Correlation of S1 and S2 with the high mol wt. polypeptides in other reports is more difficult. Collins & Alexander (1980*b*) have separated the spikes from embryonated egg-grown IBV strain M41 using Triton X-100. Two major polypeptides of mol. wt. 86 $\times$ 10^3 and 66 $\times$ 10^3 were associated with the spike-containing fraction, at least one of which (86 $\times$ 10^3 mol. wt.) was glycosylated. Recently, B. Lomniczi & J. Morser (personal communication) have reported that IBV strain Beaudette from CEK cells has a glycopeptide of 94 $\times$ 10^3 mol. wt. and a non-glycosylated polypeptide of 92 $\times$ 10^3 mol. wt. Collins & Alexander (1980*a*) have reported that both IBV strain M41 and Beaudette, grown in embryonated eggs, had a glycoprotein of mol. wt. 86 $\times$ 10^3. Correlation of the findings from several authors will require simultaneous analysis in slab gels of IBV from several laboratories. It is possible that the apparent mol. wt. of the S polypeptides will depend on the host cell in which the virus was grown, as shown for MCV (Bond *et al.*, 1979).

In conclusion, I have shown that the majority of the polypeptides in IBV preparations are host-coded. The virus-coded polypeptides of IBV from de-embryonated eggs are similar in size, except for the lack of size-class one polypeptide, and number to those of mammalian coronaviruses. The use of de-embryonated eggs should enable the rapid production of purified virus from many field isolates of IBV with the minimum of laboratory-induced variation.

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