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Coronavirus IBV: Further Evidence that the Surface Projections are Associated with Two Glycopolypeptides

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SUMMARY

The surface projections (peplomers) of avian infectious bronchitis virus (IBV) strain M41 have been separated from the nucleocapsid (N) and matrix (M) proteins by sedimentation in a sucrose gradient after virus disruption by the non-ionic detergent Nonidet P40. The peplomers comprised two glycopolypeptides of mol. wt. 90×10^3 (90K; S1) and 84K (S2), shown by analysis of differentially radiolabelled virus to be present in equimolar proportions. Polypeptides of 75K and 110K, which were detected by Coomassie Brilliant Blue staining in similar amounts to S1 and S2 in some unlabelled virus preparations, were absent from peplomer preparations and are probably host cell polypeptides. The S1 : S2 : N : M polypeptide molar ratio for IBV-M41 was approximately 1 : 1 : 6 : 15.

The avian infectious bronchitis virus (IBV) particle, like other coronaviruses, contains three major protein structures, the surface projection or peplomer (S), nucleocapsid (N) and matrix (M) proteins (Cavanagh, 1981; Siddell *et al.*, 1982). The M protein comprises a polypeptide of mol. wt. 23000 (23K) which is glycosylated to different extents to form glycopolypeptides of mol. wt. up to 36K (Stern *et al.*, 1982; Stern & Sefton, 1982; Cavanagh, 1983). A polypeptide of 50K to 54K forms the N protein (Macnaughton *et al.*, 1977). There is less agreement on the composition of the S protein. Since the presumptive S polypeptides of all coronaviruses examined have mol. wt. greater than that of the N polypeptide, the following account refers only to such IBV polypeptides.

Urea and bromelain can remove peplomers from IBV, concomitant with the removal of two glycopolypeptides S1 (90K) and S2 (84K) from [^{35}S]methionine-labelled virus grown in the chorioallantoic membrane (CAM) cells of de-embryonated chicken eggs (Cavanagh, 1981). A minor polypeptide of 75K was removed by Tween 80. The high mol. wt. polypeptides of IBV grown in embryonated eggs, and detected by Coomassie Brilliant Blue staining after polyacrylamide gel electrophoresis, have been the subject of several papers. Macnaughton & Madge (1977), using three IBV strains including IBV-M41, reported five major polypeptides of 130K, 105K, 97K, 81K and 74K of which the 105K polypeptide (p105) was present in greatest amount. Bromelain removed the peplomers concomitant with the removal of p130, p105 and p74 (Macnaughton *et al.*, 1977), and it was later concluded, on the basis of its variable occurrence and ease of removal by bromelain, that p74 was a contaminant (Macnaughton & Davies, 1980). Collins & Alexander (1980*a*) using 12 IBV strains reported major polypeptides of 180K, 107K, 98K, 94K, 86K, 75K and 60K, of which p107 and p75 were found in greatest quantity. Isolated peplomers from IBV-M41 were associated with polypeptides of 86K and 66K (Collins & Alexander, 1980*b*). Nagy & Lomniczi (1979) reported one major polypeptide of 75K and a minor polypeptide of 110K. One major polypeptide of 90K was reported by Lanser & Howard (1980).

Lomniczi & Morser (1981) compared the composition of IBV-Beaudette from embryonated eggs with that radiolabelled during replication in chick embryo kidney (CEK) cells. Virus from CEK cells had two closely migrating glycopolypeptides, referred to collectively as gp94, and a

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polypeptide of 170K; all three polypeptides were detected by both Coomassie Brilliant Blue staining and by autoradiography. In contrast, staining did not detect gp94 in virus from embryonated eggs but did detect two polypeptides of 110K and 75K. When this virus was iodinated, gp94 was detected by autoradiography. Wadey & Westaway (1981) compared the polypeptides of several strains of IBV radiolabelled in CEK and CAM cells. A major glycopolypeptide of 91K, and two minor glycopolypeptides of 185K and 210K were detected in virus from both cell types. Lastly, Stern *et al.* (1982) reported major (84K) and minor (90K and 59K) glycopolypeptides in IBV-Beaudette from CEK cells. The aim of this work was to separate IBV peplomers from the N and M proteins and to determine their polypeptide composition.

Radiolabelled IBV-M41 was prepared in pairs of de-embryonated eggs (Cavanagh, 1981); each egg received 125 μCi [^{35}S]methionine (sp. act. >800 Ci/mmol) or [^{35}S]methionine plus 165 μCi of a mixture of 15 ^{3}H-labelled amino acids (code TRK 440, Amersham International). Unlabelled virus was grown in batches of 200 11-day-old embryonated eggs which were inoculated with approx. 3·5 $\log_{10}$ median ciliostatic dose$_{50}$ of IBV-M41. After 24 h at 37 °C the eggs were chilled at 4 °C overnight. Allantoic fluid was collected, clarified at 4000 ***g*** for 30 min and the virus pelleted at 35000 ***g*** for 2·5 h. The pellet was resuspended in NET buffer (100 mM-NaCl, 1 mM-EDTA, 10 mM-Tris–HCl pH 7·4) to a vol. of 20 ml and sonicated at maximum amplitude for 10 s with the 3 mm probe of an MSE ultrasonic disintegrator. The suspension was placed on two discontinuous gradients comprising 20 ml 25% (w/w) sucrose and 5 ml 60% (w/w) sucrose in NET in a 6 × 38 MSE swing-out rotor. After centrifugation at 65000 g_{max} for 2·5 h at 4 °C, the gradients were fractionated. Fractions which contained virus at the 25/60% sucrose interphase were pooled, diluted threefold, placed on a 25 to 55% (w/w) sucrose gradient in NET and centrifuged at 50000 g_{av} for 16 h at 4 °C in a 6 × 38 rotor. Fractions of 1 ml were collected and those of density 1·16 to 1·22 g/ml were pooled, diluted to 20% (w/w) sucrose and the virus pelleted at 90000 ***g*** for 3 h at 4 °C in an MSE 3 × 25 swing-out rotor. Pelleted virus (2 to 4 mg protein) was resuspended in 1 ml 1 M-KCl or 1 M-NaCl in NET and 1 ml 4% (v/v) Nonidet P40 (NP40) in NET containing 1 M-KCl or 1 M-NaCl, followed by sonication for 2 to 3 s and incubation at 25 °C for 1 h. Undissolved material was removed by low-speed centrifugation and the supernatant placed on a 10 to 55% (w/w) sucrose gradient in NET containing 1 M-KCl or 1 M-NaCl and 0·1% NP40. After centrifugation in an MSE 6 × 38 swing-out rotor at 85000 g_{av} for 16 h at 4 °C fractions of 500 μl were collected. These were dialysed to remove KCl where appropriate prior to electrophoresis. SDS–polyacrylamide gel electrophoresis (SDS–PAGE) was performed in tubes and slabs with a 10% acrylamide resolving gel (Cavanagh, 1981). Samples were dissociated at room temperature with 2% SDS and 2% (v/v) 2-mercaptoethanol. Unlabelled markers used were phosphorylase b, bovine serum albumin and carbonic anhydrase; some phosphorylase was pre-stained with Drimarine brilliant blue K-BL (Bosshard & Datyner, 1977) and the apparent mol. wt. was 110K.

When radiolabelled IBV-M41 was dissociated with 1% NP40 and centrifuged in a 10 to 55% (w/w) sucrose gradient, no clear separation of S, N and M was obtained. When 0·1% NP40 was incorporated into the gradient, three peaks of radioactivity were detected (Fig. 1). SDS–PAGE showed that the lower peak comprised N, presumably associated with viral RNA, to form the ribonucleoprotein (RNP; Sturman *et al.*, 1980). The major component of the upper peak was the M polypeptide. The middle peak contained S1 and S2 in similar amounts and very little of any other polypeptide (Fig. 2*b*). In experiments with NP40-dissociated radiolabelled virus, monoclonal antibodies prepared against S immunoprecipitated S1 and S2 in equal amounts, confirming that the peplomers contain both S1 and S2 (A. P. A. Mockett, D. Cavanagh & T. D. K. Brown, unpublished observations).

When relatively large amounts (2 to 4 mg protein) of virus from embryonated eggs were dissociated with 2% NP40 and sedimented in sucrose gradients containing NP40, S sedimented as did the radiolabelled S, but M sedimented much further and greatly contaminated the S-containing fractions. When 1 M-KCl or 1 M-NaCl was incorporated into the dissociating solution and the gradient, as done by Collins & Alexander (1980*b*), S was obtained free of M, which sedimented only a short way into the gradient (Fig. 3*a*). Much of N was positioned between M and S; presumably the high salt concentration had dissociated N from the RNA. S1

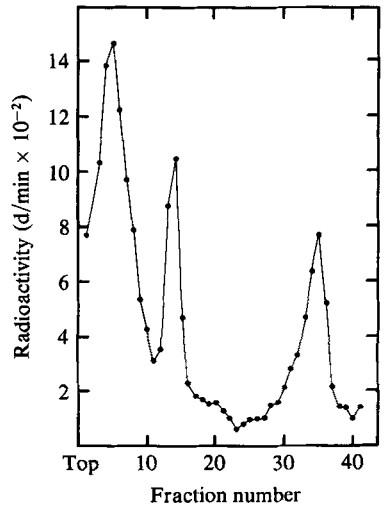

Fig. 1. Separation of IBV-M41 non-denatured proteins. [^{35}S]Methionine-labelled virus in NET was dissociated by 1% NP40 at 25 °C for 1 h. The proteins were then sedimented in a 10 to 55% (w/w) sucrose gradient in NET at 85000 g_{av} for 16 h at 4 °C in an MSE 6 × 14 swing-out rotor. Fractions of about 300 µl were collected and the radioactivity in 50 µl samples determined in a scintillation counter.

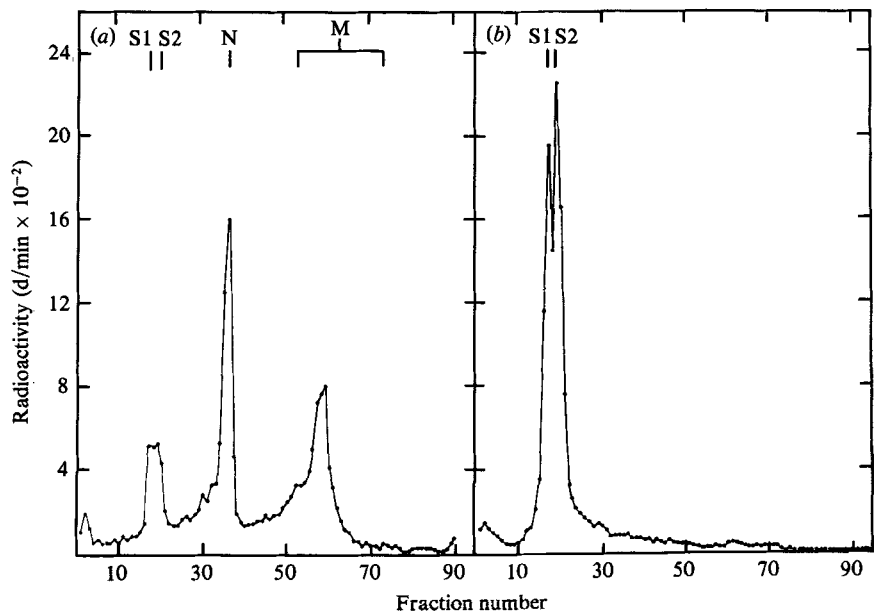

Fig. 2. SDS–PAGE of [^{35}S]methionine-labelled IBV-M41 polypeptides. (*a*) Virus used in the experiment of Fig. 1; (*b*) polypeptides present in the middle peak of Fig. 1.

and S2 were the major polypeptides of mol. wt. >54K detected in peplomer-containing fractions. Electron microscopy showed the presence of morphologically recognizable surface projections in these fractions. Fig. 3(*b, c*) shows the presence of S1 and S2 from two other preparations of S. We have examined, by SDS–PAGE, over 20 unlabelled preparations of IBV-M41 from embryonated eggs; polypeptides were visualized by Coomassie Brilliant Blue staining. All samples contained S1 and S2, and S1 was more easily detected than S2. In 75% of

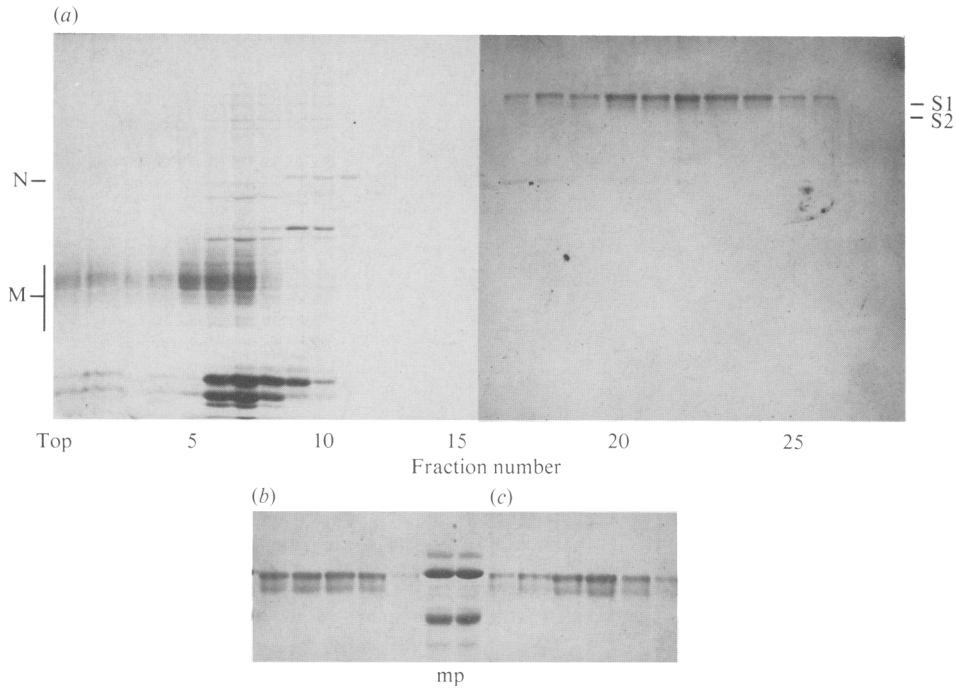

Fig. 3. SDS–PAGE in 10% acrylamide slab gels of unlabelled **IBV-M41 polypeptides,** stained with Coomassie Brilliant Blue. Virus (2 to 4 mg protein) was dissociated with 2% NP40 in 1 M-KCl in NET. (*a*) The top 28 fractions of one such gradient; (*b, c*) peplomer-containing fractions only of two other gradients [only the top part of the gels are shown in (*b*) and (*c*)]. Marker polypeptides, mp: phosphorylase b reacted with Drimarine brilliant blue K-BL, phosphorylase b, bovine serum albumin.

preparations S1 was the major polypeptide of mol. wt. greater than that of N while in the remainder the 110K polypeptide (p110) was present in similar amount to S1. In some preparations p110 and p75 were barely detectable. The S protein of Fig. 3(*b*) was derived from a virus preparation which had a greater than usual amount of p110 and p75. However, these polypeptides remained near the top of the gradient, away from the fractions containing the surface projections.

In addition to [^{35}S]methionine, IBV was radiolabelled with a mixture of 15 ^{3}H-labelled amino acids. It was assumed that the amounts of the ^{3}H label would indicate the relative amounts of the virion polypeptides. It was calculated that the ^{35}S/^{3}H d/min (disintegrations per minute) ratios for S1 and S2 were approx. 2·0 and 2·4 respectively. Thus, S2 was about 1·2-fold, i.e. 2·4/2·0, richer in methionine than S1 and therefore an S1/S2 ^{35}S d/min ratio of about 1·2 is indicative of equal amounts by weight of S1 and S2; the corresponding ratio for the S preparation of Fig. 2(*b*) was 1·3. Since the mol. wt. of the polypeptide moieties of S1 and S2 are very similar (64K and 61K respectively; Cavanagh, 1983) it follows that S1 and S2 are present in equimolar amounts.

The S1 : S2 : N : M molar ratio for IBV-M41 was 1 : 1 : 7 : 15 for one preparation and 1 : 1 : 5 : 14 for another, giving a mean ratio of 1 : 1 : 6 : 15. The mol. wt. of the polypeptide moiety of M was taken as 23K (Stern *et al.*, 1982; Cavanagh, 1983). N and M accounted for about 40% each of the virus protein by weight, while S1 and S2 each accounted for approx. 8%. Analysis of one preparation of another IBV strain (IBV-D41) gave an S1 : S2 : N : M molar ratio of 1 : 1 : 4 : 17.

Thus, these studies show that the peplomers of IBV comprise two glycopolypeptides of 90K and 84K in equimolar proportion. The polypeptides of 110K and 75K, variably detected in virus preparations, are probably host polypeptides.

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