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Coronavirus Proteins: Biogenesis of Avian Infectious Bronchitis Virus Virion Proteins

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We examined the synthesis of viral structural proteins in cultured cells infected with the avian coronavirus infectious bronchitis virus. Tryptic peptide mapping was used to determine the structural relationships of the intracellular proteins to the virion polypeptides. Pulse-chase experiments were performed to identify precursors to the virus-specific proteins. We found that the nucleocapsid protein, P51, and the small viral membrane proteins GP31, GP28, and P23 do not undergo post-translational proteolytic processing. In contrast, GP90 and GP84, the two large virion membrane proteins, were found to be produced by cleavage of a single precursor, GP155. This demonstrated that at least one coronavirus mRNA specifies two virion proteins.

The coronaviruses were so named because of the distinctive morphology of their virions. It is now clear, however, that this group of animal viruses is also distinguished by a unique mode of gene expression. Coronaviruses have large RNA genomes which are nonsegmented and single stranded. The genomes are infectious and hence of positive (messenger) polarity (11, 19). The complexity of the genome of the coronavirus avian infectious bronchitis virus (IBV) has been estimated at between 19 and 24 kilobases (12, 14, 19, 31). Thus, the IBV genome is substantially larger than other nonsegmented RNA viral genomes which have been characterized to date.

At least six viral mRNAs are synthesized in cells that are infected with avian or murine coronaviruses. The six mRNAs of IBV consist of the genome itself and five subgenomic RNAs. We found that the six IBV RNAs comprise a nested sequence set, with each species containing the seqences of every smaller mRNA. The subgenomic RNAs appear to be colinear with the genome and to be identical to 3'-terminal portions of the genome (24, 25).

Our working hypothesis has been that only the unique 5' terminus of each mRNA is translated, in a way analogous to translation of the two overlapping alphavirus mRNAs. Since IBV virions contain five distinct polypeptides (23) and IBV employs five subgenomic mRNAs, it was likely that each IBV subgenomic mRNA specifies only a single polypeptide.

We have found that IBV virions contain nine proteins (23). These include six major proteins (GP84, P51, GP36, GP31, GP28, and P23) and three minor proteins (GP90, GP59, and P14), in general agreement with other recent analyses of IBV protein composition (5, 13, 30). Peptide mapping has shown that GP90, GP84, P51, P23, and P14 are all structurally distinct (23). However, we have found that GP36, GP31, and GP28 are glycosylated forms of P23, which differ only in the extent of glycosylation (26). We designated these four proteins the "P23 family." Although the major virion proteins are apparently specified by IBV, it seemed possible that the minor proteins GP90, GP59, and P14 were contaminants derived from the host cells (23).

The protein compositions of murine coronaviruses JHM and MHV-A59 are similar to the protein composition of IBV. Murine coronaviruses contain a heterogeneous family of small glycoproteins (designated E1) which are homologous to the IBV P23 family proteins (6, 18, 22). Murine coronaviruses also contain two large glycoproteins (designated E2), which have molecular weights of approximately 90,000 and 180,000 (22, 27). It seems likely that the 180-kilodalton (kd) E2 protein is a dimer of the 90-kd form since the peptide maps of these two proteins are similar (28). Thus, in contrast to IBV virions, which contain the two large glycoproteins GP90 and GP84, murine coronavirus particles apparently contain only a single large polypeptide. In this paper we demonstrate that IBV GP84 can also be isolated as a multimer and therefore may be the IBV glycoprotein that is homologous to E2.

We investigated the synthesis of viral proteins in IBV-infected chicken embryo kidney (CEK) cells in order to ascertain the steps involved in the biogenesis of the virion proteins. We were

especially interested in determining whether any of the proteins were derived from larger precursors. We found that maturation of virion proteins P51, GP31, and P23 did not involve major post-translational proteolytic processing. However, GP90 and GP84 were found to be produced by post-translational cleavage of a single intracellular precursor, GP155. These results demonstrated that GP90 is in fact a virus-specific protein and that therefore IBV specifies three structurally distinct glycosylated polypeptides. Our results also showed that at least one coronavirus mRNA specifies two virion proteins.

MATERIALS AND METHODS

Virus and cells. The Beaudette strain (strain 42) of IBV was propagated in primary CEK cells as described previously (23, 24). Cells were incubated at 37 or 38.5°C.

Radiolabeling of intracellular proteins. CEK cultures in 60-mm dishes (Falcon Plastics) were washed once with Tris-buffered saline and infected with IBV at a multiplicity of 10 PFU/cell. After incubation for 90 min, the inoculum was replaced with 4 ml of Dulbecco-Vogt modified Eagle medium containing one-tenth the normal amount of methionine and 2% calf serum dialyzed against saline. The cultures were incubated for an additional 4.5 h, washed once with methionine-free Dulbecco-Vogt modified Eagle medium containing 2% dialyzed calf serum, and incubated with 1.0 ml of the same medium containing [^{35}S]methionine (1,200 Ci/mmol; Amersham Corp.). In pulse-chase experiments infected cultures were labeled for 15 min, washed twice with warm Dulbecco-Vogt modified Eagle medium containing 2% calf serum, and returned to the incubator for the chase period. In the experiment shown in Fig. 3A, infected cultures were labeled with 800 μCi of [^{35}S]methionine for 60 min, and the mock-infected cultures were labeled with 200 μCi of [^{35}S]methionine for 90 min. In the experiment shown in Fig. 3B, cultures were labeled with 250 μCi of [^{35}S]methionine. Intracellular proteins for peptide mapping were prepared from six infected CEK cultures, each of which was labeled for 3 h with 0.4 mCi of [^{35}S]methionine.

At the ends of the labeling periods, cell cultures were washed twice with cold Tris-buffered saline and lysed in 0.5 ml of RIPA buffer, and immunoprecipitations were performed as described previously (21). One volume of antiserum was incubated with 25 volumes of cell lysate. We determined that under these conditions antibody was in excess over viral proteins. Inactivated *Staphylococcus aureus* (Pansorbin) was obtained from Calbiochem-Behring Corp.

Preparation of virions for peptide maps. Virus particles were radiolabeled biosynthetically with [^{35}S]methionine and purified from three 160-mm dishes of CEK cells by sedimentation to equilibrium in successive sucrose and Renografin gradients, as described previously (23).

SDS-polyacrylamide gel electrophoresis. Samples were suspended in electrophoresis sample buffer (5 mM sodium phosphate, pH 7.0, 2% sodium dodecyl sulfate [SDS], 0.1 M dithiothreitol, 5% 2-mercaptoethanol, 10% glycerol, 0.4% bromophenol blue), boiled for 30 s, and analyzed by electrophoresis in discontinuous 15% acrylamide–0.09% bisacrylamide gels as described previously (21). For peptide mapping only one-half of the virion protein preparation was boiled before gel electrophoresis in order to permit recovery of protein Z. The nonboiled and boiled portions were combined and fractionated on a single gel that was 14 cm long by 2 mm thick. All other gels were 14 cm long by 1 mm thick. Analytical gels were fluorographed (3) by using preflashed Kodak X-Omat R film at −70°C. The molecular weight of GP155 was estimated by using the standards described previously (23).

Tryptic peptide mapping. Virion proteins were eluted from the preparative gel by electrophoresis (32). Intracellular proteins were eluted by homogenization of the excised gel slices (2). Eluted proteins were precipitated with trichloroacetic acid, oxidized, and digested with tolylsulfonyl phenylalanyl chloromethyl ketone-trypsin (Worthington Diagnostics) as described previously (2). Two-dimensional peptide mapping on 0.1-mm thin-layer cellulose plates (EM Reagents) was performed as described previously (2). The plates were prepared for fluorography (4) and exposed to preflashed film at −70°C.

Antisera. Virions were purified from 10 CEK cultures in 160-mm dishes as described previously (23), suspended in 300 μl of complete Freund adjuvant (Calbiochem-Behring Corp.), and injected intradermally into a rabbit in 50-μl portions. The rabbit was similarly injected a second time 5 weeks later and again after an additional 3 weeks. Antiserum was prepared from blood obtained 3 and 6 weeks after the final inoculation.

Reagent antiserum prepared from chickens infected with Massachusetts strain IBV was a gift from SPAFAS Inc.

RESULTS

Multimeric form of GP84. SDS-polyacrylamide gel electrophoresis resolves both a monomeric form and a dimeric form of the E2 glycoprotein of murine coronaviruses (22, 28). We wanted to determine whether one or both of the large IBV glycoproteins GP84 and GP90 had a similar tendency to aggregate. Our standard method of sample preparation for gel electrophoresis included incubation at 100°C. Because mild conditions favor isolation of the 180-kd form of E2 (22), we analyzed polypeptides of IBV virions disrupted at 23°C. We suspended a radiolabeled virion preparation in gel sample buffer, removed a portion, and boiled the remaining portion for 30 s. Portions of boiled (Fig. 1, lane a) and nonboiled (Fig. 1, lane b) samples were analyzed by electrophoresis. The nonboiled virions contained a large protein, designated Z, which was not observed in boiled virions, and comparatively less GP84 than the boiled virions. This showed that glycoprotein aggregates could be detected in samples of IBV virions and suggested that protein Z was related to GP84. To determine whether protein Z could be converted to GP84, we suspended a portion of gel-purified protein Z in sample buffer, boiled

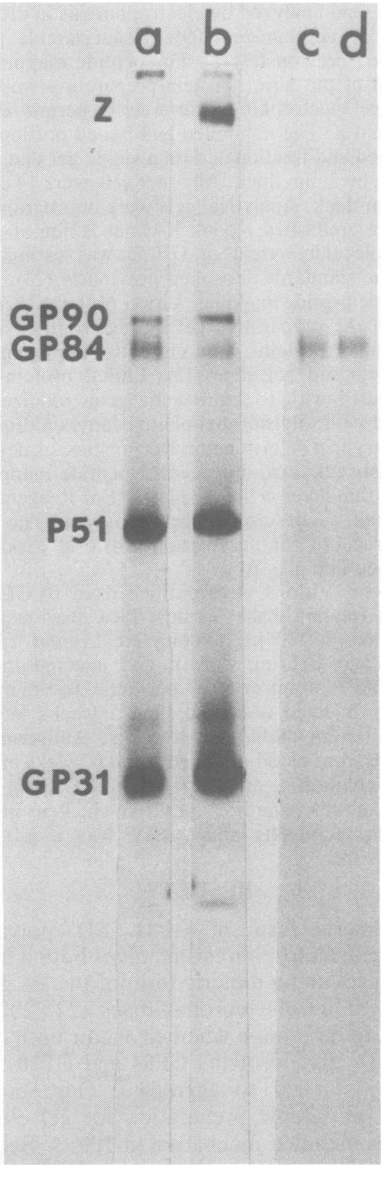

FIG. 1. Effect of boiling on the electrophoretic mobility of IBV virion proteins. Lanes a and b contained virions which were labeled biosynthetically with [^{35}S]methionine, purified as described previously (20), and suspended in electrophoresis sample buffer. One portion was incubated for 30 s at 100°C, and an equal portion was incubated at 23°C. The samples were analyzed by gel electrophoresis. A total of 25,000 cpm was applied to each lane; fluorography was for 1 day. Lanes c and d contained portions of the preparations of gel-purified protein Z and GP84 that were used for peptide mapping; these portions were suspended in sample buffer and boiled, and the samples were analyzed by gel electrophoresis. Fluorography was for 35 days. Lane a, Virions disrupted at 100°C; lane b, virions disrupted at 23°C; lane c, gel-purified protein Z; lane d, gel-purified GP84.

it, and analyzed the protein a second time on an SDS-polyacrylamide gel. The resulting band had the same mobility as GP84 prepared in the same way (Fig. 1, lanes c and d). We prepared maps of the methionine-containing tryptic peptides from protein Z, GP84, and GP90 (Fig. 2). The peptide maps of protein Z (Fig. 2A) and GP84 (Fig. 2B) were almost identical. Protein Z did not contain the major peptides of GP90 (Fig. 2C). Since protein Z could be converted to GP84 and since the peptide maps of these proteins were identical, it appears that protein Z is a multimeric aggregate of GP84, rather than an unique virion protein or a complex containing GP90 and GP84.

Cell-associated forms of IBV proteins. IBV virions contain six major proteins (GP84, P51, GP36, GP31, GP28, and P23) and three minor proteins (GP90, GP59, and P14). To identify possible precursors to these proteins, we examined the synthesis of viral proteins in infected CEK cells. IBV-infected cells were labeled with [^{35}S]methionine and lysed with RIPA buffer. The solubilized proteins were analyzed by SDS-polyacrylamide gel electrophoresis. The profile of proteins synthesized in infected cells was complex (Fig. 3A, lane g) and similar to the profile obtained for mock-infected cells (Fig. 3A, lane h), although synthesis of some virus-specific proteins was detectable. To circumvent this problem, rabbit antiserum raised against purified IBV virions was used to immunoprecipitate proteins from these lysates. Nine proteins were precipitated specifically from lysates of infected cells (Fig. 3A, lane d). Among these were six proteins (Fig. 3, open circles) which had mobilities identical to or just less than the mobilities of virion proteins GP84, P51, GP31, GP28, P23, and P14. Three of the specifically precipitated proteins did not comigrate with virion proteins. These proteins (Fig. 3, solid circles) had apparent molecular weights of 155,000, 42,000, and 40,000.

The intracellular protein just larger than virion P14 was not detected reproducibly. This protein was precipitated with both normal rabbit serum and immune serum (data not shown). However, since this protein was only detected in infected cells, it is likely that synthesis of this protein is induced by virus infection. This protein was not characterized further.

We did not detect GP90 in infected cells with the rabbit antiserum. Since it was likely that GP90 was produced in infected cells, the failure to precipitate GP90 suggested that the rabbit antiserum did not react well with the protein. This could have resulted from the fact that the purified virions used to raise this serum contained only small amounts of GP90. Virions purified by our standard procedure contain only one-tenth as much GP90 as GP84 (23). There-

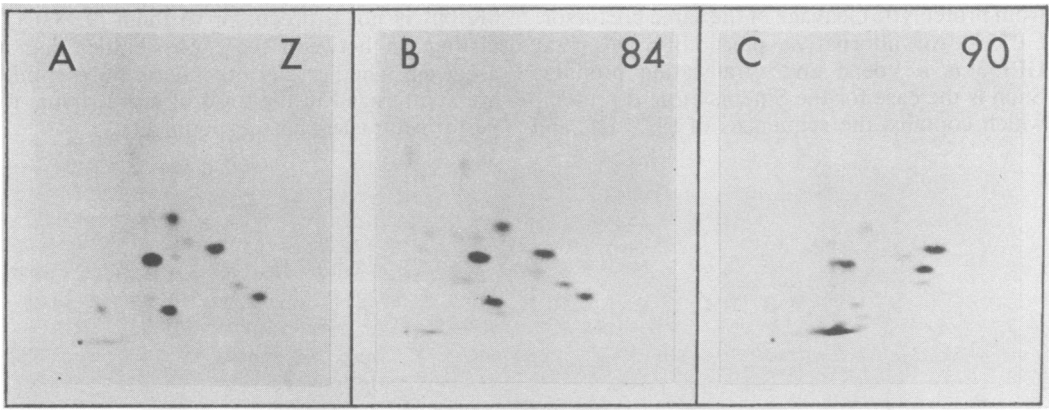

FIG. 2. Two-dimensional tryptic peptide analysis of virion proteins Z, GP84, and GP90 labeled with [^{35}S]methionine. In this figure and in Fig. 4 and 5, the origins are at the lower left. Peptides were separated on thin-layer plates by electrophoresis in the first dimension (from left to right) and ascending chromatography in the second dimension (from bottom to top). A total of 1,200 cpm was applied to each plate. Fluorography was for 21 days at −70°C. (A) Protein Z. (B) GP84. (C) GP90.

fore, we tested a serum prepared from chickens infected with IBV. Like the rabbit serum, the chicken antiserum recognized GP155, GP84, P51, GP31, GP28, and P23 (Fig. 3B, lane c). In addition, the chicken serum precipitated a 90-kd protein, which was almost certainly the intracellular form of GP90. Thus, GP90 was in fact present in infected cells but is not apparent in Fig. 3A because the rabbit serum did not recognize it.

Tryptic peptide maps of intracellular proteins. We considered it likely that the intracellular proteins with mobilities similar to those of virion proteins GP84, P51, GP31, GP28, and P23 were cell-associated forms of those proteins. To test this directly, we prepared two-dimensional maps of methionine labeled tryptic peptides from six of the virus-specific intracellular proteins. A map of the intracellular 51-kd protein was almost identical to a map of virion protein P51 (Fig. 4A and B). The chromatographic mobility of the peptide indicated by the arrowhead in Fig. 4A and B was variable (unpublished data), and it does not appear that there is a genuine difference between the two proteins. (Compare Fig. 4B with our previously published map of virion P51 [23].) Maps of the peptides from the 40-kd protein (Fig. 4C) and the 42-kd protein (data not shown) were identical to the map of P51. The 42- and 40-kd proteins may arise from degradation of P51, either within infected cells or during immunoprecipitation.

The map of virion GP31 contained only a single major polypeptide (Fig. 4E), as observed previously (23). The map of the intracellular 31-kd protein likewise contained one major peptide (Fig. 4D). The peptides comigrated when a mixture of peptides from the two proteins was examined (Fig. 4F).

The map of the intracellular 84-kd protein (Fig. 5A) was almost identical to the map of GP84 obtained from virions (Fig. 5B). Thus, our peptide mapping experiments demonstrated that these five intracellular proteins are related to virion proteins GP84, P51, and GP31.

Although we did not map the intracellular 28- and 23-kd proteins, it is almost certain that these proteins are cell-associated forms of GP28 and P23 because they can be immunoprecipitated only from infected cells. Furthermore, the intracellular 28-kd protein is glycosylated and yields the same cleavage product as bona fide GP28 when it is digested with endoglycosidase H (26).

The 155-kd polypeptide (Fig. 5C) contained all of the peptides in the maps of both GP84 (Fig. 5A) and GP90 (Fig. 5D). Maps of mixtures of peptides from the 155-kd protein and GP84 (Fig. 5E) and the 155-kd protein and GP90 (Fig. 5F) confirmed that GP155 includes the peptides which are specific to GP84 (Fig. 5, arrows pointing toward the right) and the peptides which are specific to GP90 (arrows pointing toward the left). Thus, GP155 includes the sequences of both GP84 and GP90. We had been unsure previously as to whether GP90 is encoded by IBV because this protein is not abundant in virions (23). The fact that GP90 is part of GP155 demonstrated that GP90 is a virally encoded protein and that the sequences encoding GP90 and GP84 are adjacent.

The 155-kd protein is sensitive to digestion with endoglycosidase H and is not detectable in cells which have been treated with tunicamycin (26). It is therefore a glycoprotein, and we have designated it GP155.

Processing of IBV proteins. Since GP155 contained the sequences of both GP90 and GP84, it was possible that GP90 and GP84 are derived

from proteolytic cleavage of the same precursor, GP155. An alternative possibility was that GP155 is a "dead end" translation product. Such is the case for the Sindbis virus B protein, which contains the sequences of PE2, E1, and 6K but is not a precursor to them (9, 33). To distinguish between these possibilities and to ascertain whether any other viral polypeptides are synthesized in the form of a precursor, we performed pulse-chase experiments.

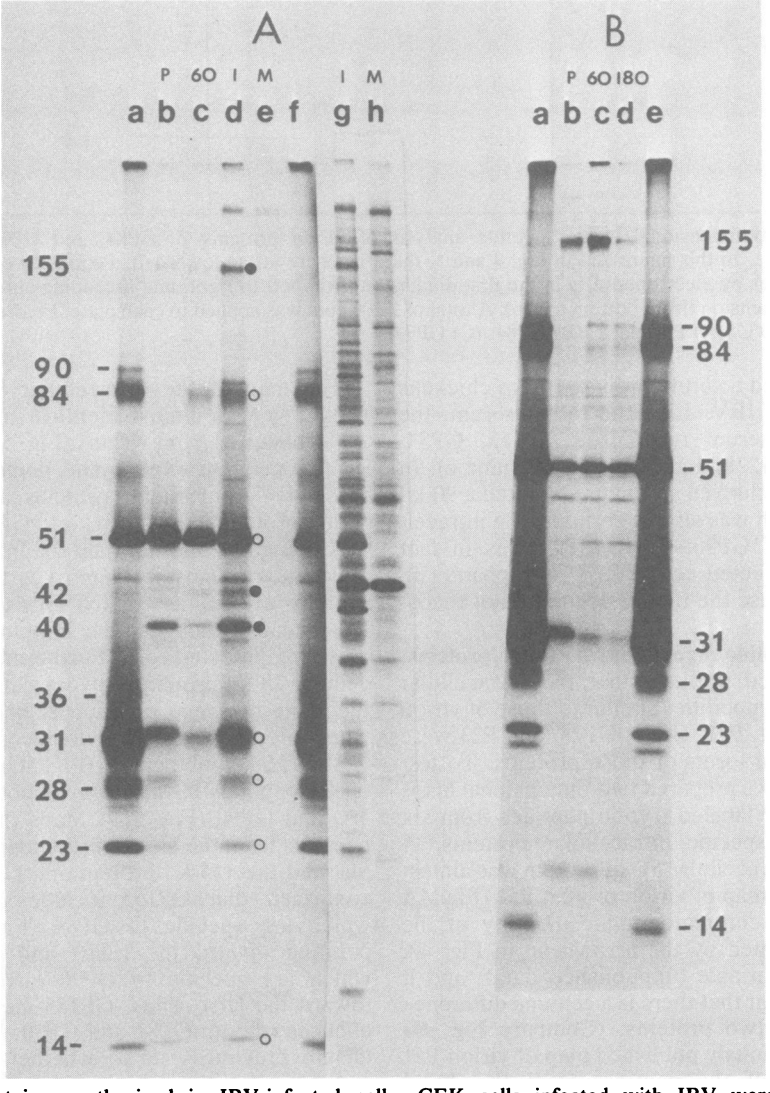

FIG. 3. Proteins synthesized in IBV-infected cells. CEK cells infected with IBV were labeled with [^{35}S]methionine as described in the text and lysed with RIPA buffer at the end of the labeling period. IBV-specific proteins were immunoprecipitated from the lysates by using rabbit anti-IBV serum (A) or chicken anti-IBV serum (B). IBV virions labeled with [^{35}S]methionine were used as markers. The labeled proteins were analyzed by SDS-polyacrylamide gel electrophoresis. (A) Composite of two different sections of one gel. Lanes a through f were exposed for 7 days, and lanes g and h were exposed for 2 h. Because more [^{35}S]methionine was used to label the infected cells than was used to label the mock-infected cells, a darker photographic print was used for lane h than for lane g. Lane a, Virions; lane b, infected cells labeled for 15 min and immunoprecipitated with rabbit antiserum; lane c, infected cells labeled for 15 min and chased for 60 min, rabbit antiserum; lane d, infected cells labeled for 60 min, rabbit antiserum; lane e, uninfected cells labeled for 90 min, rabbit antiserum; lane f, virions; lane g, infected cells labeled for 60 min, RIPA buffer lysate; lane h, uninfected cells labeled for 90 min, RIPA buffer lysate. (B) Exposed for 30 days. Lane a, Virions; lane b, infected cells labeled for 15 min, chicken antiserum; lane c, infected cells labeled for 15 min and chased for 60 min, chicken antiserum; lane d, infected cells labeled for 15 min and chased for 180 min, chicken antiserum; lane e, virions.

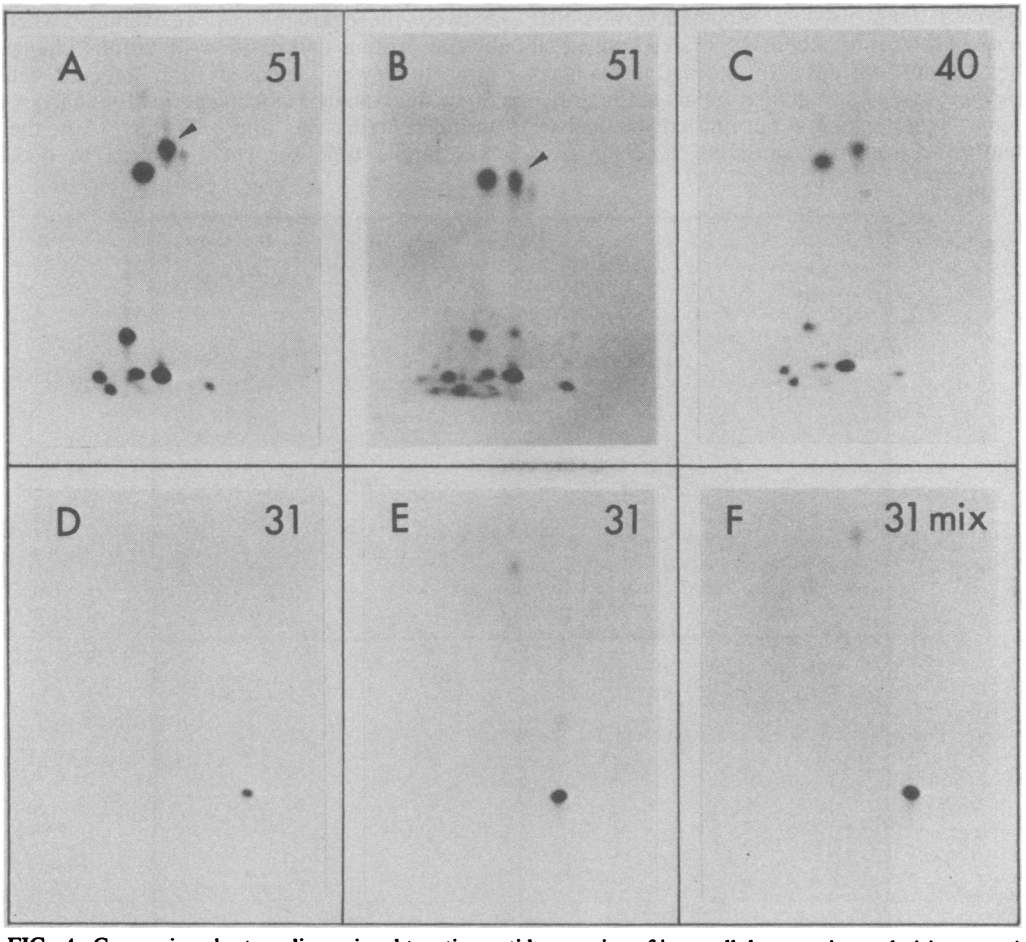

FIG. 4. Comparison by two-dimensional tryptic peptide mapping of intracellular proteins and virion proteins P51 and GP31. Proteins were labeled biosynthetically with [^{35}S]methionine, purified, and prepared for peptide mapping as described in the text. Sample activity ranged from 1,200 to 5,600 cpm. The plates were fluorographed for 5 to 8 days. (A) Intracellular 51-kd protein. (B) Virion P51. (C) Intracellular 40-kd protein. (D) Intracellular 31-kd protein. (E) Virion GP31. (F) Mixture of peptides from virion GP31 and intracellular 31-kd protein.

Infected cells were labeled for 15 min and then lysed either immediately or after a 60- or 180-min chase (Fig. 3B, lanes b through d). GP155 was evident in the pulse-labeled cells, but neither GP84 nor GP90 could be detected. GP90 and GP84 appeared only after a 60-min chase. This indicated that GP90 and GP84 are derived from a precursor. Since GP155 includes the sequences of GP90 and GP84, can be labeled in a short period of time, and is the only virus-specific protein larger than GP90 and GP84, it must be an obligatory precursor to GP90 and GP84.

GP155, P51, GP31, GP28, and P23 were all evident immediately after a 15-min labeling period. This is most obvious in Fig. 3A, lane b. In other experiments these proteins could be detected in cells labeled for as little as 5 min (data not shown). The presence of labeled GP31 and GP28 in cells labeled for 5 min showed that if these proteins are generated by post-translational processing of P23, then such processing must occur within 5 min of release of P23 from polysomes.

Pulse-labeled GP155, GP31, and GP28 all decreased slightly in apparent molecular weight during a 60-min chase (Fig. 3A, lanes b and c). These changes were discernible as soon as 30 min after the onset of labeling (data not shown) and could result either from proteolytic cleavage of the proteins or from processing of the oligosaccharides of the proteins.

Loss of GP90 from virions. Cleavage of GP155 should produce equimolar amounts of GP90 and GP84. Therefore, why GP84 was much more abundant than GP90 in virions remained to be

explained. One possible explanation was that the two proteins are not incorporated with equal efficiency into virions. An alternative explanation was that the two glycoproteins are incorporated in equal amounts, but that GP90 is subsequently lost from virus particles. To distinguish between these possibilities, we examined whether the protein composition of virions changed after they were released from infected cells. Since our standard labeling period for analysis of virion proteins was approximately 12 h, there was ample time for such changes to occur.

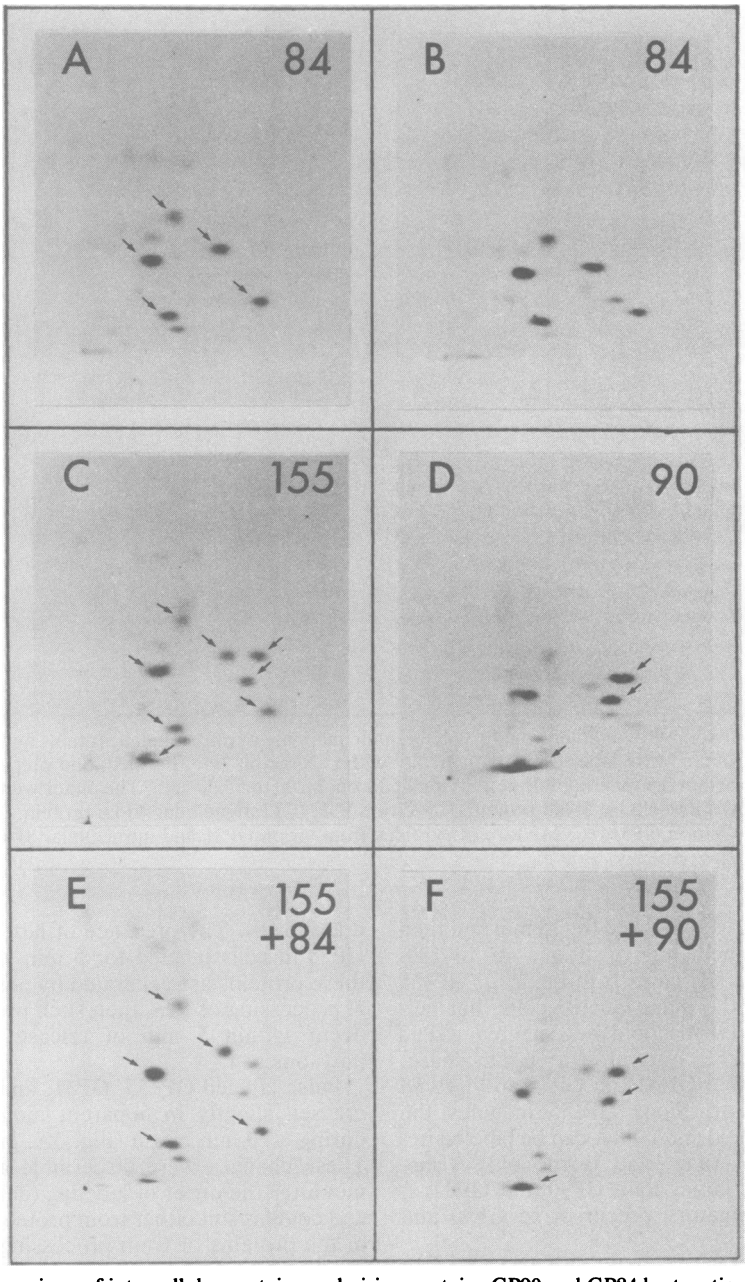

FIG. 5. Comparison of intracellular proteins and virion proteins GP90 and GP84 by tryptic peptide mapping. Sample activities ranged from 700 to 1,200 cpm. Exposures were for between 5 and 60 days. The peptides marked with arrows were specific to GP84 (arrows pointing toward the right) or specific to GP90 (arrows pointing toward the left). (A) Intracellular 84-kd protein. (B) Virion GP84. (C) GP155. (D) Virion GP90. (E) Mixture of peptides from intracellular 84-kd protein and GP155. (F) Mixture of peptides from virion GP90 and GP155.

Therefore, we examined the composition of virions labeled for a significantly shorter period. Virions harvested after labeling for 3 h contained nearly equal labeled amounts of GP90 and GP84 (Fig. 6, lane a). This demonstrated that GP90 was incorporated into virions efficiently and suggested that GP90 was subsequently lost from released virus particles. To investigate this further, a portion of the medium from which these virions had been purified was incubated for an additional 18 h at 37°C without cells. Virions prepared from the incubated medium contained comparatively little GP90. The amount of GP84 normalized to the amount of P51 decreased only slightly (Fig. 6, lane b). Thus, it appeared that GP90 is efficiently packaged into virus particles but is either degraded or released from extracellular virions.

DISCUSSION

We found that infection of CEK cells with IBV resulted in the synthesis of the polypeptides found in virions and at least three nonvirion polypeptides. Two of the nonvirion polypeptides, with apparent molecular weights of 42,000 and 40,000, appear to be degradation products of the nucleocapsid protein P51. The third nonvirion protein, GP155, was found to be the precursor to virion glycoproteins GP90 and GP84.

Tryptic peptide mapping revealed that GP155 contained the sequences of GP90 and GP84. This demonstrated for the first time that GP90 is a virus-encoded protein and suggested that GP155 might be a precursor to GP90 and GP84. GP90 and GP84 were not labeled with a short pulse, but appeared as labeled species after a 60-min chase. Because GP155 was the only large virus-specific polypeptide detected in pulse-labeled cells, it must be the precursor to both GP90 and GP84.

Previous work had suggested that the large glycoproteins of the avian coronaviruses are quite different from those of the murine coronaviruses. IBV virions contain two structurally distinct large glycoproteins, GP84 and GP90 (5, 23). In contrast, the murine coronaviruses contain a single large glycoprotein species, E2, which is characterized by a tendency to form dimeric and even larger aggregates (22, 28). We found that a discrete multimer of GP84, protein Z, was present in preparations of IBV virions which were disrupted by SDS and a reducing agent without heating. Under the same conditions, however, no aggregates of GP90 were detectable. Since protein Z has a lower electrophoretic mobility than fibronectin (unpublished data), its molecular weight is probably in excess of 240,000. This suggests that each molecule of protein Z contains three molecules of GP84 and

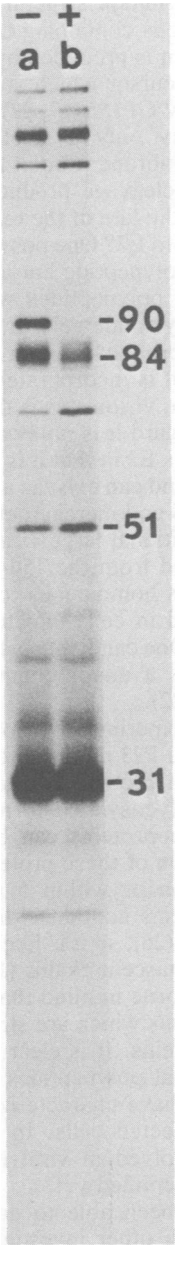

FIG. 6. Loss of GP90 from virions incubated at 37°C. Infected 60-mm CEK cultures were labeled with 200 μCi of [^{35}S]methionine for 3 h, and the medium was harvested and stored at −70°C. A 100-μl portion of the medium was thawed and incubated at 37°C for 18 h. This sample and an equal portion of medium which had not been incubated were diluted with 50 mM Tris (pH 7.4)–100 mM NaCl–1 mM EDTA, and virions were pelleted by sedimentation at 85,000 × *g* for 3 h at 4°C in an SW50.1 rotor. Fluorography was for 4 days. Lane a, Virions from nonincubated medium; lane b, virions from incubated medium.

might imply a trimeric structure for the virion surface projections containing GP84.

The E2 protein is produced by cleavage of an intracellular precursor which is virtually identical in size to IBV GP155 (17, 22). This raises the question of why only a single large murine coronavirus membrane protein has been identified. A second cleavage product must be produced. What is the fate of the remaining portion of the precursor to E2? One possibility is that E2 is not a single polypeptide but actually consists of two different polypeptides which comigrate on SDS-polyacrylamide gels. It is also possible that the second product is either degraded inside the cell or that it is incorporated into, but then rapidly lost from, virions, as is the case for IBV GP90. In this regard it is noteworthy that GP84 of IBV resembles E2 in that it is stably associated with virions and can exist as a multimer in the presence of SDS and mercaptoethanol. We predict that an additional large virus-specified glycoprotein derived from the 150-kd intracellular protein, which is homologous to GP90 of IBV, will be identified in cells infected with murine coronaviruses. One candidate is the 65-kd glycoprotein which is a minor component of JHM virus particles (22).

Pulse-chase experiments showed that P51, GP31, GP28, and P23 do not undergo extensive post-translational proteolytic processing. GP31 and GP28 are glycosylated forms of P23 (26). Since these glycoproteins can be labeled in 5 min, glycosylation of these proteins must occur during translation or within 5 min afterward. These two proteins contain N-linked oligosaccharide residues (26), so it is likely that they are glycosylated as nascent chains (16, 20).

It should be borne in mind that we examined only those proteins which are structurally related to virion proteins. It is clear, however, that nonstructural viral polypeptides other than the three which we have characterized are synthesized in IBV-infected cells. In particular, the polypeptides involved in viral RNA synthesis have not been identified yet.

We have not been able to detect GP155 in virions. Although other investigators have detected polypeptides larger than 90 kd in IBV virions (13, 30), the identity of these large proteins is uncertain; they could be GP155 or aggregates such as protein Z. From our data it appears that cleavage of GP155 occurs before virions exit from the cells. Since coronaviruses bud into vesicles inside infected cells (1, 7, 8, 10, 19), there is undoubtedly a delay between budding and release of virus particles from the cell. Therefore, it is difficult to determine the exact timing of the cleavage of GP155 with respect to virion formation.

IBV P51 and P23 can be synthesized by in vitro translation of IBV mRNAs A and C, respectively (23). Thus, these proteins are primary translation products both in vivo and in vitro. This indicates that mRNAs A and C each encode a single mature polypeptide. In contrast, GP90 and GP84 are both produced by cleavage of a single precursor, GP155. A non-glycosylated form of this precursor can be synthesized by in vitro translation of mRNA E (unpublished data). Therefore, although two coronavirus mRNAs specify single polypeptides, at least one mRNA specifies two virion proteins.

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