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# Structural proteins of human respiratory coronavirus OC43

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## Summary

The human respiratory coronavirus OC43 was grown on a human rectal tumor cell line and was isotopically labeled with amino acids, glucosamine, and orthophosphate to analyze virion structural proteins. Four major protein species were resolved by electrophoresis and many of their properties were deduced from digestion studies using proteolytic enzymes. The four proteins are: (1) A 190 kDa protein, the presumed peplomeric protein, that was glycosylated and proteolytically cleavable by trypsin into subunits of 110 and 90 kDa. The subunits each represent a different amino acid sequence on the basis of peptide mapping; (2) a 130 kDa protein that was glycosylated and behaved as a disulfide-linked dimer of 65 kDa molecules. It is the apparent virion hemagglutinin on the basis of digestion studies with trypsin, bromelain and pronase; (3) a 55 kDa nucleocapsid protein that was phosphorylated; (4) a 26 kDa matrix protein that was glycosylated. The 190, 130, 55 and 26 kDa species can therefore be designated P, H, N and M, respectively. They exist in molar ratios of 4 : 1 : 33 : 33, and are calculated to be present at the rate of 88, 22, 726, and 726 molecules per virion, respectively.

human respiratory coronovirus OC43, structural proteins

## Introduction

Coronaviruses are estimated to cause approximately 20% of upper respiratory disease in humans (Larsen et al., 1980; McIntosh et al., 1970). Human respiratory coronaviruses are found as either one of two antigenic types: those related to human coronavirus OC43 (viruses that could originally be grown only in tracheal organ culture), and those related to human coronavirus 229E (viruses that could be

isolated in tissue culture) (McIntosh et al., 1970). A rigorous characterization of the structural proteins of human respiratory coronaviruses may therefore require a separate focus on each antigenic subgroup.

All coronaviruses studied in detail to date appear to share a common genome structure, namely, a single-stranded, non-segmented, polyadenylated, infectious RNA molecule of between 5 and 7 MDa in size (Siddell et al., 1982). Most coronaviruses, but not all, can be described as having three major unique structural protein species: a 150–200 kDa peplomeric glycoprotein (often posttranslationally proteolytically modified to two subunits of around 60–100 kDa), a 45–55 kDa internal nucleocapsid phosphoprotein, and a 23–30 kDa envelope-associated, matrix-like glycoprotein (Siddell et al., 1982; Sturman and Holmes, 1983). This description fits the well-characterized mouse hepatitis coronavirus A59 (Sturman, 1977), the porcine transmissible gastroenteritis coronavirus (Garwes and Pocock, 1975), and the avian infectious bronchitis virus (Stern and Sefton, 1982), representatives of three of the four major coronavirus antigenic subgroups (Pedersen et al., 1978; Wege et al., 1982). A fourth major structural protein, a 130–140 kDa glycoprotein comprised of disulfide-linked, 65 kDa subunits, is found on the hemagglutinating mammalian coronaviruses, including the hemagglutinating porcine encephalomyelitis coronavirus, the bovine enteric coronavirus, and the human coronavirus OC43, all antigenically closely related to MHV A59 (Callebaut and Pensaert, 1980; Hogue et al., 1984; King et al., 1982). The 140 kDa glycoprotein is the apparent virion hemagglutinin on the bovine coronavirus (King et al., 1985).

We have previously reported that HCV OC43 has an analogous antigenic counterpart to each of the four major structural proteins of the bovine enteric coronavirus (Hogue et al., 1984). In this report we describe studies that further characterize the structural proteins of HCV OC43 by showing the following. The 190 kDa protein is glycosylated and is cleavable by trypsin into subunits of 110 and 90 kDa, each of which appears to be comprised of a different amino acid sequence as determined by peptide mapping. The 130 kDa protein is a dimer of disulfide-linked glycoproteins of 65 kDa, and is the apparent virion hemagglutinin as judged from digestion studies with trypsin, bromelain and pronase. The 55 kDa protein is phosphorylated. The 26 kDa protein is glycosylated and readily aggregates to form dimeric molecules of around 38 kDa. The 190, 130, 55 and 26 kDa species can therefore be designated P, H, N, and M for peplomer protein, hemagglutinin, nucleocapsid protein and matrix protein, respectively. They exist on the virion in molar ratios of 4:1:33:33, and are calculated to be present at the rate of 88, 22, 726 and 726 molecules per virion, respectively.

## Materials and Methods

### Cells

The human rectal carcinoma cell line HRT-18 (Tompkins et al., 1974) was grown as monolayers in Dulbecco modified Eagle medium containing 50 $\mu$g of gentamicin per ml and 5% fetal bovine serum (Sterile Systems, Inc.).

*Virus*

Human coronavirus OC43 (HCV OC43) was obtained from S. Weiss, University of Pennsylvania, Philadelphia. The virus had been passaged 7 times in human embryonic tracheal organ culture followed by 15 passages in suckling mouse brain. The virus was cloned in our laboratory by two successive isolations from single plaques. Cloned virus was passaged at a multiplicity of $< 0.1$ pfu/cell and viral stocks were prepared from passages 3 through 8 by infecting cells at a multiplicity of 0.8 pfu/cell. Viral titers ranging from $10^6$ to $10^7$ pfu/ml were obtained in stock preparations.

*Plaque assays*

HCV OC43 was assayed on HRT-18 cell monolayers in 35 mm dishes (Costar). Cells were used between passages 7 and 14 during which time they grew as a glassy-smooth monolayer. Virus plaques were most visible when cells were in this condition at the time of starting the plaque assay. Virus was adsorbed for 1.5 h at 37°C and cells were overlaid with medium containing 2% fetal calf serum and 0.5% agarose. Plaques were counted 4 days after infection and were either visualized directly or by hemadsorption using a 0.4% mouse erythrocyte suspension.

*Hemagglutination assay*

The hemagglutination assay was performed as described previously (King and Brian, 1982).

*Isotopic labeling of viral polypeptides*

Confluent monolayers of cells grown in 150 cm$^2$ flasks were rinsed twice with Earle's balanced salt solution before being infected with virus at a multiplicity of approx. 1 pfu/cell. After 1.5 h of adsorption at 37°C, the inoculum was removed and 15 ml of the appropriate medium and the isotope were added. All isotopes were obtained from ICN Pharmaceuticals, Inc. Viral polypeptides were labeled by adding 400 $\mu$Ci of $^3$H-labeled essential amino acid mixture (150–200 mCi/mg) per flask in medium containing 10% normal essential amino acid concentration and 2% fetal calf serum. Viral glycoproteins were labeled by adding 400 $\mu$Ci of [$^3$H]glucosamine (5–15 Ci/mmol) per flask to medium containing 5% fetal calf serum. Viral phosphoproteins were labeled by adding 500 $\mu$Ci of [$^{32}$P]orthophosphate in medium containing 10% normal phosphate concentration and 20% fetal calf serum.

*Virus purification*

Infected supernatant fluids were clarified by centrifugation at $7\,500 \times g$ for 10 min, and virus was concentrated by sedimentation onto cushions of 60% (wt/wt) sucrose at $90\,000 \times g$ for 2 h. Concentrated virus was isopycnically sedimented on 18 ml continuous 60–20% (wt/wt) sucrose gradients for 4.5 h at $90\,000 \times g$ in a Sorvall AH-627 rotor and gradients were fractionated using an ISCO gradient fractionator. Virus sedimented to a buoyant density of 1.18–1.20 g/cm$^3$, as determined from a coincidental peak of hemagglutinating activity and radioactivity, and collected virus was diluted in TMEN (50 mM Tris acid maleate, pH 6.0, 0.1 M

NaCl, 1 mM EDTA) and pelleted at 90 000 × $g$ for 2.5 h in a Sorvall AH-627 rotor. All sucrose solutions were made in TMEN and all steps were carried out at 4°C.

*Phenol extraction of ^{32}P-labeled viral proteins*

[32]P-labeled virus was purified, pelleted, dissolved in 1% SDS in 0.5 ml of TNE (50 mM Tris, pH 9.0, 0.1 M NaCl, 1 mM EDTA), and mixed in a Vortex blender for 5 min at 20°C with an equal volume of TNE-saturated phenol. The phenol phase was separated by centrifugation at 1 000 × $g$ for 3 min, and reextracted twice with an equal volume of TNE. Viral proteins were precipitated from the phenol by adding 5 volumes of chilled methanol and 100 $\mu$g of bovine serum albumin as carrier and storing overnight at −20°C. The precipitate was collected by centrifugation and washed twice with methanol. The pellet was suspended in 0.5 ml of TMN (50 mM Tris acid maleate, pH 6.0, 0.1 M NaCl) by gentle bath sonication. One portion was digested with 20 units of $T_1$ RNAse (Calbiochem) and 40 $\mu$g pancreatic RNAse (Sigma) per ml for 1 h at 37°C, and a second portion with 1 mg proteinase K (Beckman) per ml for 1 h at 37°C, prior to electrophoresis.

*Treatment of purified virus with proteolytic enzymes*

For trypsin treatment, purified virus was incubated with 20 $\mu$g, 1 mg, or 2 mg trypsin (type XI, Sigma) per ml in TMN for 30 min at 37°C as described in the figure legends. When testing for loss of hemagglutinability, concentrations of 1 mg and 2 mg per ml were used. The digestion reactions were stopped by adding an equal volume of 8 mg lima bean trypsin inhibitor (Sigma) per ml, and the virus was immediately pelleted. For bromelain treatment, a modified procedure of Compans (Compans et al., 1970) was used. Purified virus was incubated with 1.3 mg bromelain (Sigma) per ml in TMN containing 5 mM dithiothreitol for 1 h at 37°C, and the virus was immediately sedimented isopycnically. For pronase treatment, purified virus was incubated with 1.0 mg pronase (type VI from *Streptomyces grisius*, Sigma) per ml in TMN for 1 h at 37°C, and the virus was immediately sedimented isopycnically.

*Polyacrylamide gel electrophoresis*

The discontinuous buffer gel system of Laemmli was used (Laemmli, 1970). Samples were dissolved in sample treatment buffer (0.125 M Tris-hydrochloride, pH 6.8, 4% SDS, 5 M urea) and treated at 100°C for 2 min immediately before electrophoresis. Electrophoresis was performed in slab gels (0.75 × 100 × 160 mm) using concentrations of 7%, 8%, or 5–15% polyacrylamide. A 1 cm stacking gel of 3% polyacrylamide was used. Electrophoresis was carried out using a current of 15 mA per slab gel. Gels were analyzed by autoradiography or fluorography after Enhance or Enlightening (New England Nuclear Corp.) treatment. Dried gels were exposed to preflashed Kodak X-Omat R film by the method of Laskey and Mills (Laskey and Mills, 1975). For molar ratio determination, lanes from the dried gel were cut into 1 mm sections, rehydrated in 0.1 ml water, digested overnight in 3% Protosol (New England Nuclear Corp.) at 37°C, and counted in 120 ml Econofluor (New England Nuclear Corp.).

*Chymotryptic peptide mapping*

Recovery of proteins from dried gels and chymotryptic peptide mapping followed published procedures (Huang et al., 1985) except that dried gels had been prepared for fluorography (Cleveland, 1983). Proteins labeled with a mixture of [$^3$H]amino acids were recovered from gel slices that had been rinsed in freshly prepared 1% ammonium bicarbonate and minced. Minced gels were incubated at 37°C, 14 h, with constant rocking in a mixture of 30 $\mu$g/ml chymotrypsin (Sigma) in 1% ammonium bicarbonate. At 14 h, fresh chymotrypsin was added and samples were rocked for another 4 h. Eluted peptides were clarified in an Eppendorf centrifuge tube and the supernatant was freeze-dried. Peptides were dissolved in a mixture of 100 $\mu$l formic acid–methanol (4 parts formic acid, 1 part methanol) and 50 $\mu$l performic acid (prepared by mixing 9 parts of formic acid with 1 part of 30% hydrogen peroxide at room temperature for 1 h), and incubated at 0°C for 3 h. Peptides were lyophilized, dissolved in distilled water, lyophilized again and analyzed by HPLC chromatography on a Waters Associates C18 Bondapack column (0.39 cm × 30 cm) using a linear gradient of acetonitrile (10% to 60% in 35 min) in 0.1% trifluoroacetic acid with a flow rate of 1.2 ml/min.

*Antiserum preparation*

Proteins from 1.2 mg of purified BCV were separated by SDS-polyacrylamide gel electrophoresis and used to prepare antiserum in rabbits as described previously (Hogue et al., 1984). Monoclonal antiserum to the gp26 protein of HCV OC43 was a kind gift from J. Fleming, University of Southern California, Los Angeles.

*Immunoblotting*

The method of Towbin et al. (Towbin et al., 1979) as modified by Burnette (Burnette, 1981) was used. Proteins were electrophoretically transferred from polyacrylamide gels to nitrocellulose paper for 2.5 h using a voltage gradient of 100 V in a Hoeffer electroblotting apparatus. Nitrocellulose paper with bound proteins was incubated with preimmune serum or antiserum diluted 1 : 10 in buffer containing 50 mM Tris-hydrochloride (pH 7.4), 150 mM NaCl, 5 mM EDTA, 0.05% w/v NP40, and 0.25% w/v gelatin. The nitrocellulose wash buffer consisted of 150 mM NaCl, 5 mM EDTA, 50 mM Tris-HCl (pH 7.4), 0.25% gelatin, 0.5% Triton, and 0.1% SDS.

## Results

*Identification of the glycosylated and phosphorylated structural proteins of HCV OC43*

When purified [$^3$H]amino acid-labeled HCV OC43 was analyzed by electrophoresis, four distinct protein bands were routinely resolved (Fig. 1, lanes 1 and 2, reprinted from Hogue et al., 1984). These had $M_r$s of 190000, 130000, 55000, and 26000. The 26000 $M_r$ protein was characteristically observed as a group of 3 or 4 closely migrating bands and the 130000 $M_r$ species behaved as a disulfide-linked dimer of 65000 $M_r$ subunits (Fig. 1 and Hogue et al., 1984). In some virus preparations, but not all, either one or more of three additional proteins were
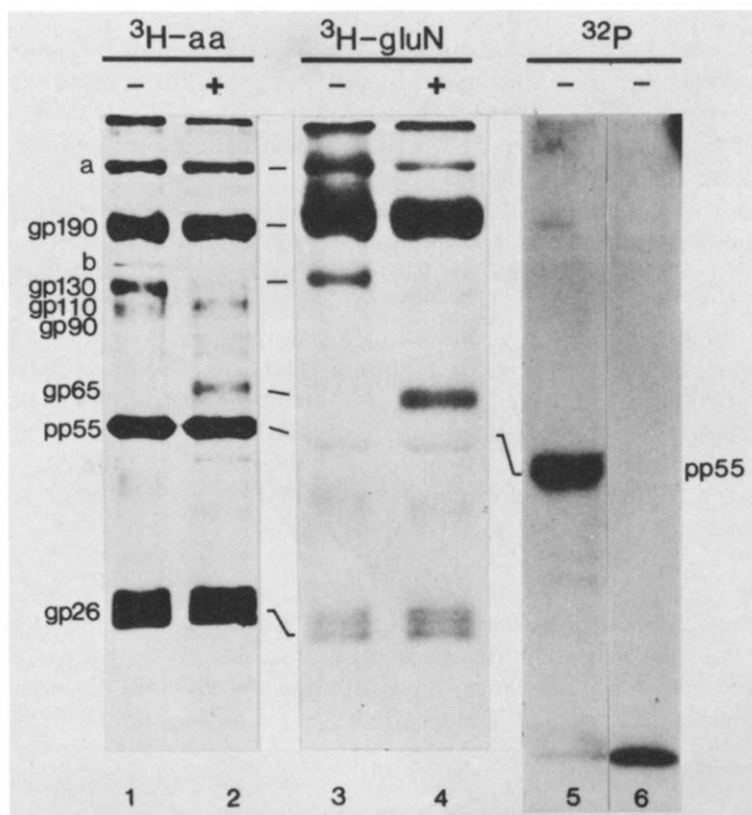
Fig. 1. Electrophoretic analysis of HCV OC43 polypeptides. HCV OC43 labeled with either [³H]amino acids (lanes 1 and 2) or [³H]glucosamine (lanes 3 and 4) was electrophoresed in the absence (lanes 1 and 3) or presence (lanes 2 and 4) of 2% 2-mercaptoethanol. ³²P-labeled virus was treated with RNAse (lane 5) or proteinase K (lane 6), as described in the text, and electrophoresed in the absence of 2% 2-mercaptoethanol. Gel acrylamide concentrations were a gradient of 5–15% for lanes 1, 2, 3, and 4, and 8% for lanes 5 and 6. $M_r$ estimates were determined from measurements against radioactive protein molecular weight standards obtained from New England Nuclear Corp. (not shown).

present in highly variable amounts. These were a protein of $M_r$ approx. 240 000, identified as "a" in Figs. 1, 2, and 4, a protein of $M_r$ approx. 160 000 identified as "b" in Figs. 1 and 4, and a protein of $M_r$ approx. 38 000 identified as "c" in Figs. 2 and 4. The 240 kDa species immunoreacted with antiserum specific for gp190 (Fig. 2) suggesting it is an aggregate or possibly a native polymeric form of gp190. The 160 kDa species, present only in the absence of reducing agent, immunoreacted with antiserum specific for the nucleocapsid phosphoprotein suggesting it is an aggregate, possibly a trimer, of this molecule (Hogue et al., 1984). The 38 kDa species reacted by immunoblotting with polyclonal antiserum specific for gp26 (Fig. 2 and Hogue et al., 1984), as well as with monoclonal antiserum specific for gp26 to yield an essentially identical pattern (data not shown). This protein is therefore the apparent
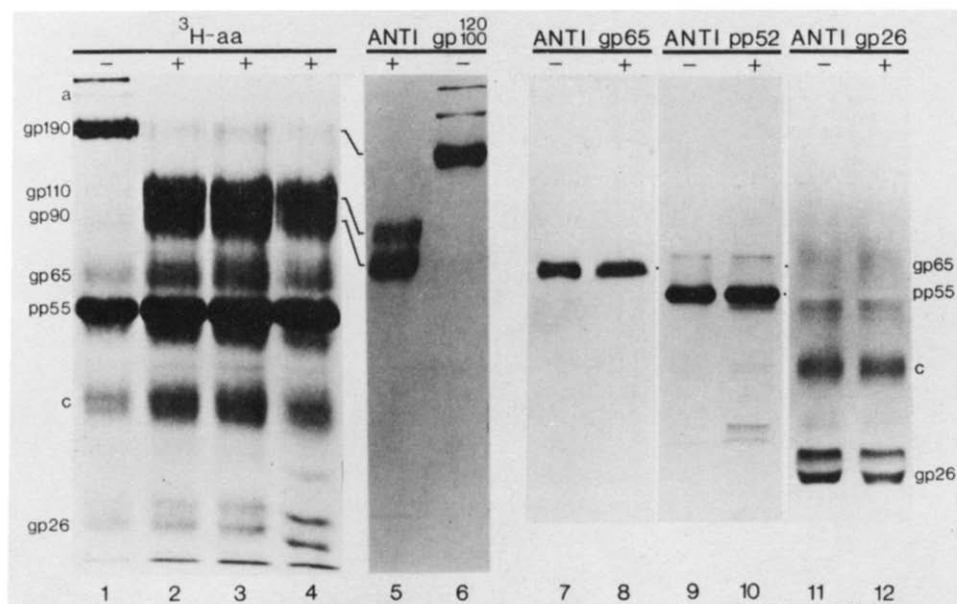
Fig. 2. Effects of trypsin on the proteins of HCV OC43. Purified HCV OC43 labeled with [³H]amino acids (lanes 1 through 4) was treated with trypsin at 20 µg (lane 2), 1 mg (lane 3) or 2 mg (lane 4) per ml under the conditions described in the text and electrophoresed on an 8% polyacrylamide gel. Purified unlabeled virus (lanes 5 through 12) was incubated with trypsin at 1 mg/ml (lanes 5, 8, 10 and 12) or without trypsin (lanes 6, 7, 9, and 11), electrophoresed on a 7% polyacrylamide gel, electrotransferred to nitrocellulose, and immunoblotted with the indicated antisera prepared against the polypeptides of the bovine coronavirus (Hogue et al., 1984). Electrophoresis in all cases was done in the presence of 2% 2-mercaptoethanol.

second rung in a ladder of multimeric forms of a self-aggregating gp26 molecule.

To examine further the identity of individual protein species, bands were removed from gels and recovered proteins were analyzed by chymotryptic peptide mapping (Fig. 3). For these studies, the amount of material in species "a" was insufficient to yield a map. From the data depicted in Fig. 3 we conclude that the 190, 130 (i.e., the combined 65 kDa subunits), 55 and 26 kDa species are each unique. Furthermore, the maps of the 160 and 38 kDa species show peptides in common with the 55 and 26 kDa species respectively, thus supporting our earlier conclusions based on immunologic data that these are multimeric aggregates of discrete polypeptide species (Hogue et al., 1984).

To determine which of the polypeptides are glycosylated, virus labeled with [³H]glucosamine was purified and analyzed by gel electrophoresis. The $M_r$s of species with 190 000, 130 000 (and its reduced subunits of 65 000), and 26 000 were each glycosylated and were thus respectively designated gp190, gp130 (gp65), and gp26 (Fig. 1). The variable $M_r$ 240 000 species was also glycosylated (species a, Fig. 1), a property consistent with it being an aggregate or another polymeric form of gp190. To determine which of the polypeptides are phosphorylated, ³²P-labeled
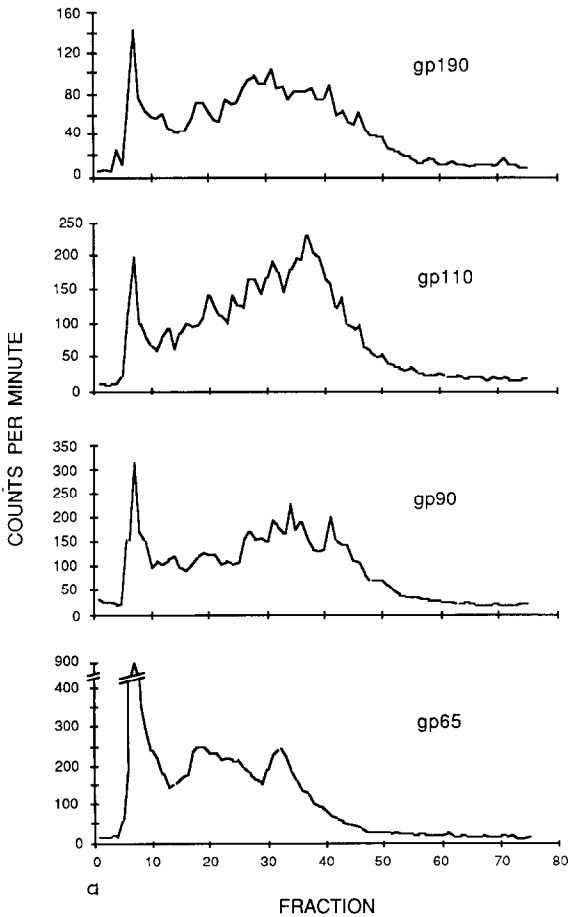
Fig. 3. Separation of the [$^3$H]amino acid-labeled chymotryptic peptides from virion structural proteins by HPLC. Proteins were recovered from gels, digested, and chromatographed as described in Materials and

virus was extracted with phenol, methanol precipitated, treated with RNAse to remove any contaminating RNA, and analyzed by gel electrophoresis. The $M_r$ 55 000 polypeptide was the only species autoradiographically observed after RNAse treatment, although its presence was completely destroyed by proteinase K treatment (Fig. 1), and it was therefore designated pp55.

*Trypsin cleaves the 190 kDa glycoprotein into unique subunits of 110 kDa and 90 kDa*
In a series of immunoblotting experiments designed to examine antigenic homology between the proteins of HCV OC43 and the bovine coronavirus, we learned that antiserum prepared against the 120 and 100 kDa proteins of the bovine coronavirus usually reacted only with the 190 kDa glycoprotein of HCV OC43 and
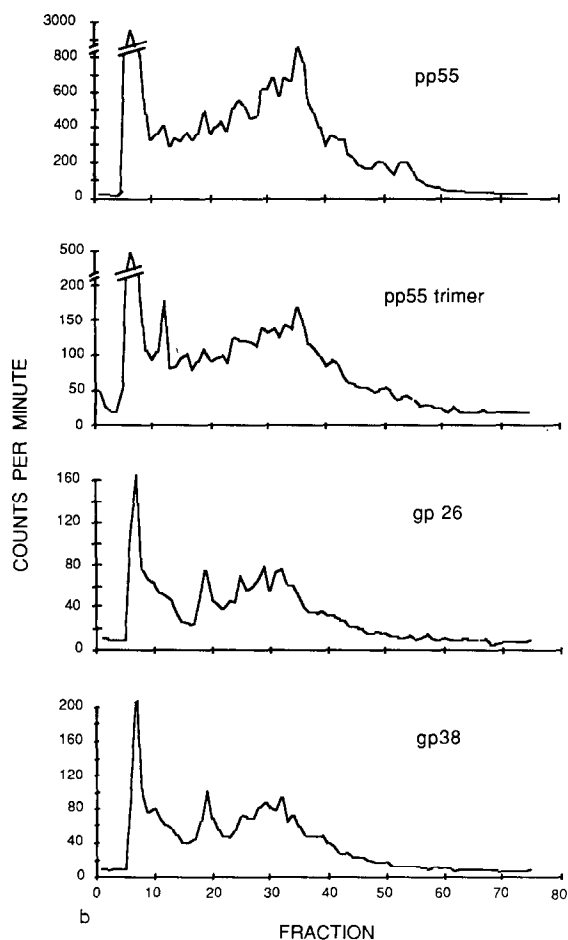
Methods. Fractions of 0.5 ml were collected and counted in 3 ml Scintiverse (Fisher).

the 240 kDa species when present (Hogue et al., 1984, and Fig. 2). In some preparations of virus a minor reactivity was also observed with HCV OC43 proteins of 110 and 90 kDa. (These proteins electrophoretically comigrate with the 120 and 110 kDa species of the bovine coronavirus, but were assigned different $M_r$s as a result of more rigorous measurements.) When tested against purified bovine coronavirus, this antiserum reacted with the 120 and 100 kDa proteins and also with a 190 kDa protein, a minor species (Hogue et al., 1984). It also reacted with gp180 (E2) of the mouse hepatitis virus A59 and its subunits of $M_r$ 90 000 (Hogue et al., 1984; Sturman and Holmes, 1977). These data together suggested a possible multimeric relationship between the HCV OC43 gp190 and the 110 and 90 kDa proteins. We therefore tested the hypothesis that HCV OC43 gp190 is structurally analogous to the MHV E2 glycoprotein, that is, has a trypsin-sensitive cleavage site. Figure 2, lanes 1 through 4, illustrate the conversion of gp190 to apparent subunits

of 110 kDa and 90 kDa by trypsin treatment of [$^3$H]amino acid-labeled virus, and, in lanes 5 and 6, the confirmation of this conversion by identification with polypeptide-specific antiserum. Under the digestion conditions used, complete or nearly complete conversion to the 110 kDa and 90 kDa subunits was observed when concentrations of 20 $\mu$g trypsin per ml or higher were used (Fig. 2 and data not shown). The $M_r$ 240 000 species "a" is also no longer present after trypsin treatment, and this lends further support to the notion that it is an aggregate or polymeric form of gp190.

To determine whether the 110 and 90 kDa subunits are unique, each was isolated, digested with chymotrypsin and the peptide profiles compared by HPLC. Although the profiles of both are contained within that of gp190, the gp110 and gp90 profiles are very dissimilar (Fig. 3) and we therefore conclude that they, at least in large part, are comprised of unique amino acid sequences.

*Trypsin and bromelain fail to remove the 130 kDa glycoprotein and also fail to completely destroy hemagglutinating activity*

Insensitivity of the bovine coronavirus 140 kDa glycoprotein to removal by bromelain led to the conclusion that it is the virion hemagglutinin (King et al., 1985). To examine whether the same pattern of protease sensitivity also exists for a second hemagglutinating coronavirus, purified HCV OC43, either unlabeled or radiolabeled with [$^3$H]amino acids, was subjected to digestion with trypsin, bromelain or pronase, repurified, and examined for hemagglutinability and protein composition. Digestion of HCV OC43 with trypsin or bromelain reduced hemagglutinability by 30% and 50% on average, respectively, as determined by comparing the total number of hemagglutinating units in the peak fractions with a parallel sample of untreated virus (data not shown). These are the mean values of 2 experiments for trypsin and 4 experiments for bromelain. Pronase completely destroyed hemagglutinating activity.

TABLE 1

MOLAR RATIOS AND ESTIMATED NUMBERS OF HCV OC43 STRUCTURAL PROTEINS [a]

| Protein | Proposed designation | % of label incorporated | Molar ratios | Number of molecules per virion [c] |
|---|---|---|---|---|
| gp190 | P | 21.1 ± 3.5 | 4 | 88 |
| gp130 | H | 3.6 ± 0.6 | 1 | 22 |
| pp55 | N | 50.8 ± 3.0 | 33 | 726 |
| gp26 [b] | M | 24.5 ± 2.1 | 33 | 726 |

[a] Determined by counting the radioactivity in 1 mm sections of a gel lane as described in the text. Electrophoresis was performed in the absence of 2-mercaptoethanol. Data represent an average of five separate experiments and variability is shown as ± one standard deviation of the mean.

[b] Counts for the 38 kDa species are combined with those for gp26 since we conclude this species to be an aggregate of gp26.

[c] Assuming a mean virion mass of 112 000 kDa as determined from a virion sedimentation coefficient of 390 (Hierholzer et al., 1972), and a virion protein content of 70%.
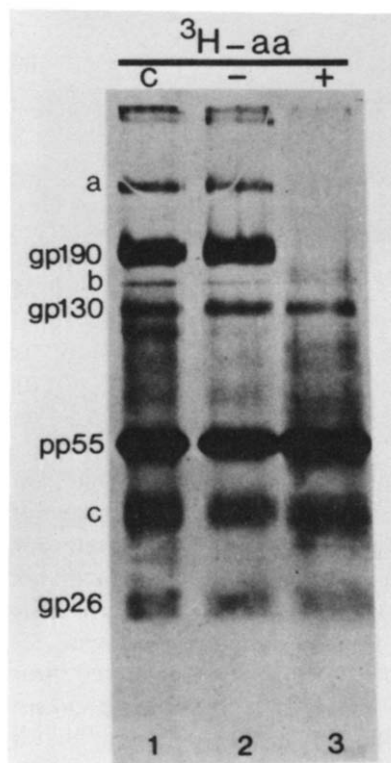
Fig. 4. Effects of bromelain on the proteins of HCV OC43. Purified HCV OC43 labeled with [³H]amino acids was divided into equal portions and analyzed by electrophoresis and fluorography after the following treatments. No further treatment (lane 1); incubated in the absence of bromelain and repurified isopycnically (lane 2); incubated with bromelain as described in the text and repurified isopycnically (lane 3). Electrophoresis was done in the absence of 2% 2-mercaptoethanol and gels were gradients of 5–15% acrylamide.

As with bovine coronavirus, retention of hemagglutinating activity following protease treatments correlated with maintenance of an apparently unaltered gp130 (or gp140 in the case of the bovine coronavirus). Gp190, as described above, was converted almost quantitatively to its subunits by trypsin at all concentrations (Fig. 2, lanes 1–4), and was apparently completely removed by bromelain (Fig. 4). It therefore probably plays no role in hemagglutination. The role of gp26 is less clear. Certainly treatment with trypsin at 2 mg/ml resulted in an increased migrational rate of the gp26 proteins suggesting proteolytic cleavage of an external moiety has occurred (Fig. 2, lane 4). Bromelain, however, appears to have little effect on gp26 (Fig. 4, lane 3). Pronase, as in the case of the bovine coronavirus, completely removed the gp190 and gp130 species (data not shown).

Taken together, these results suggest that gp130 is the virion hemagglutinin of HCV OC43.

*Molar ratios of virion polypeptides*

The molar ratios of virion polypeptides were determined and an estimate of the number of polypeptide molecules per virion was made as described in Table 1.

## Discussion

We conclude that HCV OC43 is comprised of four major structural proteins: three glycoproteins of 190, 130, and 26 kDa that can be designated P, H and M because of their apparent respective roles as peplomeric protein, hemagglutinin and matrix protein, and one phosphoprotein of 55 kDa designated N because of its apparent role as the nucleocapsid protein. Chymotryptic peptide analysis suggests that each is a unique species, supporting our earlier conclusions that were based on immunochemical data (Hogue et al., 1984).

Our studies further show that the virion gp190 of HCV OC43, like the 180 kDa glycoprotein (E2) of MHV A59, possesses a trypsin sensitive site, the cleavage of which yields two subunits that are unidentical as judged from $M_r$s and chymotryptic peptide maps. Responses of the 110 and 90 kDa subunits to 2-mercaptoethanol suggest that there are disulfide linkages both within each subunit and between the subunits. Firstly, there was retarded migration of both subunits following 2-mercaptoethanol treatment (data not shown). Secondly, when trypsin-treated virus was repurified in the presence of 2-mercaptoethanol prior to protein electrophoresis, the relative amount of gp110 was greatly reduced (data not shown) suggesting that it had been released from its anchorage to the virion via disulfide bonds to gp90. The exact nature of the multimeric form of gp190, the 240 kDa species "a" in Figs. 1, 2, and 4, was not determined. It is possibly a multimer of gp190 or some aggregated combination of one or both of the gp190 subunits. Precedence for the aggregation of just one subunit to yield discrete high $M_r$ multimers on polyacrylamide gels is found in the behavior of the smaller subunit of the IBV peplomeric protein (Stern and Sefton, 1982). We observe that the chymotryptic profiles of the HCV OC43 gp110 and gp90 subunits are both contained within the profile of gp190 (Fig. 3). A profile of their sum, however, suggests there is proportionally more·gp110 in the subunit form following trypsin treatment (data not shown).

The degree of posttranslational cleavage of the MHV A59 E2 protein is a function of host cell type (Frana et al., 1985). Although we have not investigated the susceptibility of the HCV OC43 gp190 in different cell types, we do find for the closely related bovine coronavirus, also grown on HRT cells, that the homologous protein exists primarily as the cleaved products. This suggests a critical structural difference exists between the proteins of the two viruses. We have never observed fusion of cells during infection with HCV OC43 or BCV so we therefore cannot test the role of gp190 cleavage as has been done for MHV A59 (Sturman and Holmes, 1984; Sturman et al., 1985).

Three of the HCV OC43 proteins identified in our study are very similar in size to the three major proteins of $M_r$ 190000, 52000, and 24000 reported by Schmidt and Kenny (Schmidt and Kenny, 1982) for the same virus. Because gp130 comprises

less than 4% of the total virus protein on a mass basis (Table 1), it is less likely than the other structural proteins to be revealed by staining and may explain why it was not visualized in the studies by Schmidt and Kenny. Hierholzer et al. (Hierholzer et al., 1972) reported six proteins for OC43 ranging from $M_r$ 191 000 to 15 000. Of these, the $M_r$ 191 000, 60 000, 47 000, and 30 000 species correspond approximately in size to the major proteins we observed when virus was electrophoresed in the presence of 2-mercaptoethanol (Fig. 1). Interestingly, in the studies by Hierholzer et al., bromelain did completely destroy the hemagglutinability of the virus for chicken erythrocytes. While other important variables differ between their experiments and ours, namely growth of HCV OC43 in suckling mouse brain and purification of virus in sodium tartrate gradients, differences in hemagglutinability following bromelain treatment may, in addition, reflect a difference in receptor molecules between chicken and mouse erythrocytes.

The virion mass of HCV OC43 has been estimated from its sedimentation coefficient to be $112 \pm 5 \times 10^6$ daltons (Hierholzer et al., 1972). From this mass and from determination of the molar ratios of individual proteins, we calculate the number per virion of gp190(P), gp130(H), pp55(N), and gp26(M) to be 88, 22, 726, and 726 respectively (Table 1). These numbers are only estimates since error could arise from the assumptions we make for calculation. We assume, for example, that the virion is 70% protein, that the particles are homomorphic, that no virion proteins are lost during purification, and that all virus proteins are accounted for in the bands we choose to quantitate from the gel. We noted in our analysis of the bovine coronavirus (King and Brian, 1982) that the 52 kDa nucleocapsid phosphoprotein was present on the virion in equal numbers to the 26 kDa glycoprotein, and suggested from this ratio that there may be a direct molecular interaction between these species. A similar ratio between the pp55 and gp26 proteins of HCV OC34 lends further support to this hypothesis.

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