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Proteolytic Cleavage of the E2 Glycoprotein of Murine Coronavirus: Activation of Cell-Fusing Activity of Virions by Trypsin and Separation of Two Different 90K Cleavage Fragments

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In the murine coronavirus mouse hepatitis virus, a single glycoprotein, E2, is required both for attachment to cells and for cell fusion. Cell fusion induced by infection with mouse hepatitis virus strain A59 was inhibited by the addition of monospecific anti-E2 antibody after virus adsorption and penetration. Adsorption of concentrated coronavirions to uninfected cells did not cause cell fusion in the presence of cycloheximide. Thus, cell fusion was induced by E2 on the plasma membrane of infected 17 Cl 1 cells but not by E2 on virions grown in these cells. Trypsin treatment of virions purified from 17 Cl 1 cells quantitatively cleaved 180K E2 to 90K E2 and activated cell-fusing activity of the virions. This proteolytic cleavage yielded two different 90K species which were separable by sodium dodecyl sulfate-hydroxyapatite chromatography. One of the trypsin cleavage products, 90A, was acylated and may be associated with the lipid bilayer. The other, 90B, was not acylated and yielded different peptides than did 90A upon limited digestion with thermolysin or staphylococcal V8 protease. Thus, the cell-fusing activity of a coronavirus required proteolytic cleavage of the E2 glycoprotein, either by the addition of a protease to virions or by cellular proteases acting on E2, which was transported to the plasma membrane during virus maturation. There is a striking functional similarity between the E2 glycoprotein of coronavirus, which is a positive-strand RNA virus, and the hemagglutinin glycoprotein of negative-strand orthomyxoviruses, in that a single glycoprotein has both attachment and protease-activated cell-fusing activities.

The coronavirus mouse hepatitis virus strain A59 (MHV-A59) contains two large glycoproteins of molecular weights 180,000 and 90,000 (e.g., 180K and 90K glycoproteins, respectively) which exhibited identical radiolabeling patterns and tryptic peptides (38, 40). Recent studies indicated that both forms of glycoprotein, designated E2, shared antigenic determinants (43, 45). The present investigation was undertaken to explore the relationship between 90K and 180K E2 species and to determine the role of proteolytic cleavage in coronavirus cytopathology and infectivity.

Several lines of evidence suggest that E2 is made as a large glycoprotein and that the 90K species results from proteolytic cleavage. In MHV-A59-infected 17 Cl 1 cells labeled for short pulses with radiolabeled amino acids, E2 appears first as a 150K to 180K glycosylated species (11, 30, 35). In vitro translation of mRNA 3 from MHV-A59 in frog oocytes yields a 150K glycosylated form of E2 (31). In the presence of tunicamycin, a small amount of nonglycosylated 120K E2 is found in infected cells (24, 30, 34).

Proteolytic cleavage of peplomeric glycoproteins has been demonstrated for several other enveloped RNA viruses and often plays an important role in the function of the viral glycoprotein (16). For example, the F glycoprotein of Sendai virus (32), the hemagglutinin glycoprotein of influenza virus (19), and the HN glycoprotein of Newcastle disease virus (23) all undergo proteolytic cleavage in infected cells. This cleavage is required for the activation of cell-fusing or neuraminidase activity (17, 18, 22, 32).

In this paper, we demonstrate that cleavage of the 180K E2 glycoprotein by trypsin in vitro or by cellular proteases yields two 90K species which can be separated and which differ in acylation and in the products of limited proteolytic digestion. Cleavage of 180K E2 to 90K species is required for the activation of cell-fusing activity of the E2 glycoprotein.

(This work was presented in part at the June 1983 EMBO Workshop on Molecular Biology and Pathogenesis of Coronaviruses, Zeist, The Netherlands [42].)

MATERIALS AND METHODS

Cells and virus. The 17 Cl 1 line of spontaneously transformed BALB/c 3T3 cells and the L2 line of C3H murine fibroblasts were propagated as previously described (44). MHV-A59 was produced in 17 Cl 1 cells and radiolabeled from 1 to 24 h postinfection in medium containing ^{3}H-amino acids (5.0 μCi/ml), ^{14}C-amino acids (1.0 μCi/ml), [^{3}H]fucose (2.5 μCi/ml), [^{3}H]palmitic acid (20.0 μCi/ml), or [^{35}S]methionine (3.0 μCi/ml) (purchased from New England Nuclear). Purification of released virions was carried out by precipitation with polyethylene glycol at 4°C, suspension in 0.05 M HEPES (*N*-2-hydroxyethylpiperazine-*N*^{1}-2-ethanesulfonic acid) buffer (pH 7.1) containing 0.85% NaCl (HEPES-saline), and sedimentation into a 30 to 50% (wt/wt) discontinuous sucrose gradient containing HEPES-saline, followed by equilibrium sedimentation in a 20 to 50% (wt/wt) sucrose gradient in TMEN 6.5 buffer (50 mM Tris-maleate, 1 mM EDTA, 100 mM NaCl [pH 6.5]) as described by Sturman et al. (43).

Antibodies. Preparation and characterization of monospecific rabbit antisera directed against isolated E1 and E2 glycoproteins of detergent-disrupted MHV-A59 virions have been described by Sturman et al. (43).

SDS-PAGE. For sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), viral proteins were ana-

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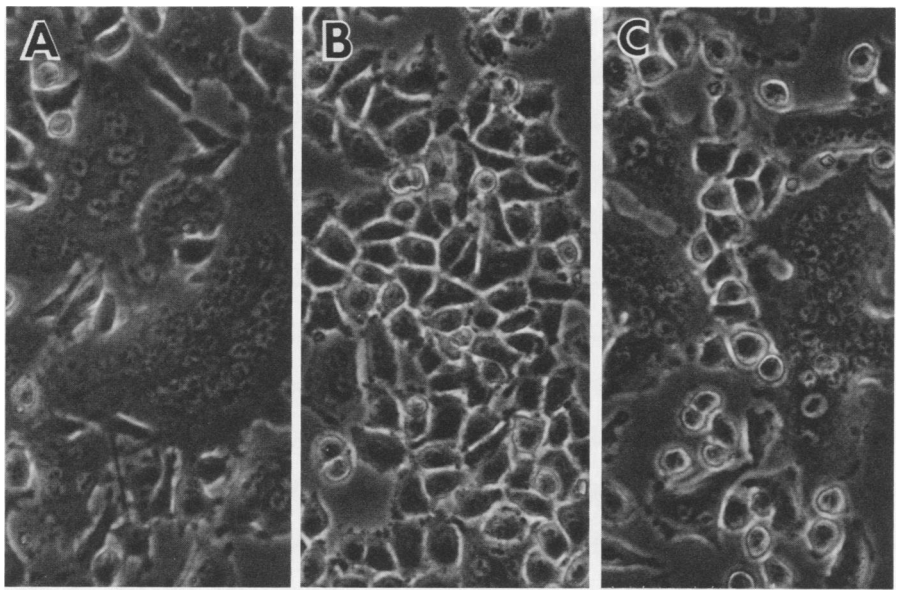

FIG. 1. Effects of monospecific anti-E1 and anti-E2 antisera on MHV-A59-induced cell fusion. L2 cells infected at a multiplicity of 3 PFU per cell show significant cell fusion after 12 h (A). Monospecific antibody against the E2 peplomer or spike glycoprotein (B) markedly inhibits cell fusion when added to the culture from 2 h after infection, but monospecific antiserum against the E1 membrane glycoprotein (C) does not prevent virus-induced cell fusion.

lyzed on 10% cylindrical polyacrylamide gels by a Laemmli buffer system and fractionated with a Gilson gel fractionator (38).

Protease treatment of virions. Purified radiolabeled virions harvested directly from a 20 to 50% linear sucrose gradient were treated with a 100-μg/ml concentration of trypsin containing tolylsulfonyl phenylalanyl chloromethyl ketone (Sigma) for 30 min at 37°C in TMEN 6.5 buffer, followed by incubation for 30 min at 4°C with a 100-μg/ml concentration of soybean trypsin inhibitor (Worthington). The virus was then repurified by centrifugation through a 20 to 50% linear sucrose gradient in TMEN 6.5 buffer. Virus was concentrated by sedimentation through 2 ml of 20% sucrose in an SW27 rotor at 24,000 rpm for 2.5 h at 4°C, and the pellet was suspended in 0.05 M HEPES-saline (pH 6.5). Control virus was treated similarly, except that trypsin was omitted. Viral proteins were analyzed by SDS-PAGE or separated by hydroxapatite chromatography.

Chromatographic separation of viral proteins. Viral proteins were separated by hydroxapatite chromatography in SDS as described by Moss and Rosenblum (21) with slight modification. Hydroxapatite (Bio-Gel HT or HTP from Bio-Rad Laboratories) was washed with 0.01 M sodium phosphate (pH 6.4)–0.1% SDS, with or without 1 mM dithiothreitol (Sigma). Columns (0.9 by 20 cm) were poured over a 0.5-cm layer of Sephadex G25 (Pharmacia, fine grade). Samples, 0.25 to 15 ml in volume, were applied and washed with 5 ml of 0.01 M phosphate containing 0.1 to 1% SDS with or without 1 mM dithiothreitol. The column was developed with a linear phosphate gradient (0.2 to 0.5 M or 0.15 to 0.45 M), and 1-ml fractions were collected at flow rates of 1 to 5 ml/h obtained by gravity. Refractive indices were measured on every 10th fraction, and the phosphate concentration determined by using a standard curve.

Limited proteolysis of isolated viral proteins. Fractions from the 90A or 90B peaks from hydroxapatite columns were chosen which were not contaminated with other viral proteins as demonstrated by SDS-PAGE of the pooled fractions. These were concentrated by using Millipore CX-30 filter units. ^{3}H-amino-acid-labeled 90A (22 μg per sample) and ^{14}C-amino-acid-labeled 90B (55 μg per sample) containing equal counts per minute of radiolabel were mixed in 0.07 to 0.10 M sodium phosphate buffer (pH 6.4) containing 0.1% SDS. An equal volume of 0.126 M Tris hydrochloride (pH 6.7), containing 2 or 10 μg of thermolysin (Boehringer-Mannheim) was added, and the mixture was incubated for 1 h at 37°C. Equivalent mixtures of 90A and 90B were incubated similarly with 0.5 or 2.0 μg of protease V8 from *Staphylococcus aureus* (Miles). Before the addition of V8 protease, Nonidet P-40 was added to the mixture to a final concentration of 2%, as this enzyme was active in 0.05% SDS only in the presence of Nonidet P-40. After incubation for 1 h at 37°C, the reactions were stopped by the addition of SDS to a final concentration of 2% and heated at 100°C for 2 min before analysis by SDS-PAGE. Prestained molecular weight protein markers (Bethesda Research Laboratories) were added to the sample mixtures immediately before electrophoresis.

RESULTS

Role of E2 in cell fusion. Cell fusion is frequently a prominent feature of coronavirus infection in vivo and in vitro. After infection of an L2 cell monolayer with MHV-A59, small syncytia were seen between 6 and 7 h. By 10 to 20 h after infection (Fig. 1A), most cells had fused to form syncytia containing tens to hundreds of nuclei. These giant syncytia detached from the substrate and died.

To demonstrate that a viral structural protein is responsible for coronavirus-induced fusion of L2 cells, we added antivirion antibody or preimmune sera to cultures after virus adsorption and observed the monolayers for evidence of syncytia formation. Cell fusion was markedly inhibited by antivirion antibody but not by preimmune serum (data not shown). This suggested that the fusion of L2 cells was induced by a viral structural protein which was present on the surface of the infected cells and accessible to antibody.

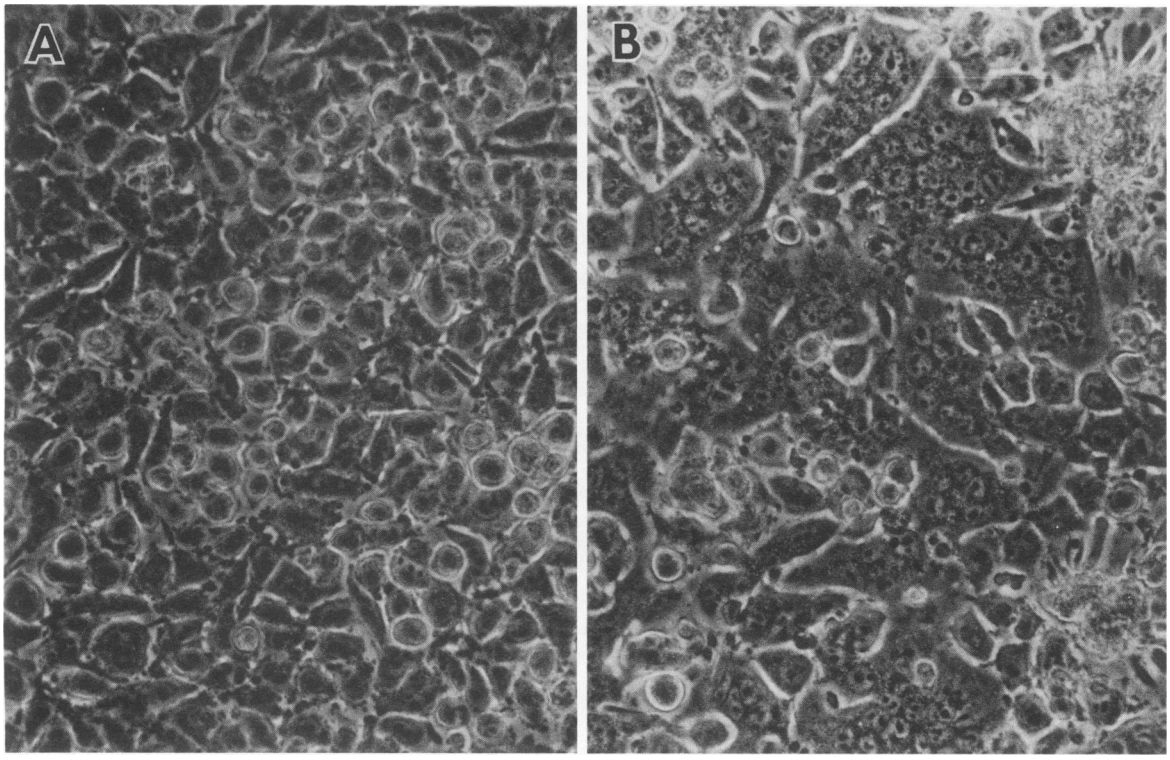

FIG. 2. Cell fusion by direct action of concentrated MHV-A59. L2 cells infected at a multiplicity of 500 PFU per cell did not exhibit cell fusion within 2 h (A). However, if the virions had been previously treated with trypsin, cell fusion occurred within 75 min (B). Virions were allowed to adsorb to prechilled L2 cells at 4°C in serum-free Eagle minimal essential medium at pH 7.0 to 7.6. After 45 to 60 min, the temperature was raised to 37°C by the addition of a 10-fold excess of warm Eagle minimal essential medium, and the cells were maintained at 37°C (pH 7.0 to 7.6).

To identify the viral glycoprotein responsible for cell fusion, we added monospecific antiserum directed against purified E1 or E2 to L2 cell monolayers 2 to 4 h after inoculation with MHV-A59. Cell fusion was markedly inhibited by anti-E2 antibody (Fig. 1B) but not by anti-E1 antibody (Fig. 1C). Thus, E2 glycoprotein on the surface of infected cells was responsible for coronavirus-induced cell fusion.

Proteolytic activation of fusion induced by concentrated virus. Many other cell-fusing viruses can induce fusion either as a result of virus replication (fusion from within) or through the direct action of concentrated inactivated virus or viral glycoprotein adsorbed to the plasma membrane of susceptible cells (fusion from without). MHV-A59 virions released from 17 Cl 1 cells were used for these studies. The yield of virus from these cells was much higher than that from other cell lines (8). Assays for rapid fusion were done in L2 cells, as these were more susceptible to fusion during MHV infection than 17 Cl 1 cells. We found that concentrated virus from 17 Cl 1 cells, when adsorbed to L2 cells at 4°C and then heated to 37°C, did not induce fusion from without (Fig. 2A). In L2 cell cultures, cell fusion was not observed after the addition of more than 500 PFU of MHV-A59 per cell grown in 17 Cl 1 cells until the end of the latent period (6 h), and no fusion was detected in the presence of a 1-μg/ml concentration of cycloheximide. However, if virions released from 17 Cl 1 cells were pretreated with trypsin, extensive fusion of L2 cells was observed by 75 min at 37°C (Fig. 2B). This rapid response to trypsin-treated virions also occurred when protein synthesis was inhibited with cycloheximide. MHV-A59 virions from 17 Cl 1 cells contained approximately equal amounts of 180K and 90K E2 (Fig. 3). Treatment of virions with trypsin quantitatively converted the 180K form of E2 to the 90K species and did not alter the other viral structural proteins E1 or N (Fig. 3; 40). The minor peak in fraction 44 represents a dimer of E1. No virus protein is removed from virions by trypsin treatment (40). These experiments showed that proteolytic cleavage of the 180K form of E2 by trypsin to the 90K species activated the cell-fusing activity of the E2 glycoprotein on virions. Rapid cell fusion was not produced in 17 Cl 1 cells inoculated with the same trypsin-activated, concentrated virus preparation. Thus, there are significant differences in the sensitivities of various uninfected cells to coronavirus-induced cell fusion.

Cleavage of E2 by trypsin did not enhance MHV infectivity. Virions released from 17 Cl 1 cells exhibited only a twofold increase in virus infectivity after treatment with trypsin at 10 μg/ml for 50 min at 37°C (40). Treatment of virions with a 10-fold higher concentration of trypsin (100 μg/ml) did not change the virus titer (93% of the control ± 43% standard deviation; mean of 24 experiments).

Separation of the two 90K fragments produced by trypsin cleavage of 180K E2. To determine whether 90K E2 is a single protein species or two different species which comigrated, we attempted unsuccessfully to separate two species of 90K E2 by isoelectric focusing or SDS-PAGE of virions labeled with [^{3}H]fucose, which labels only E2, and [^{35}S]methionine, which labels all of the viral structural proteins. However, with hydroxyapatite chromatography, two fucose-labeled E2 species could be separated readily from purified trypsin-treated, SDS-disrupted virions (Fig. 4). Analysis by SDS-PAGE revealed that both of these peaks

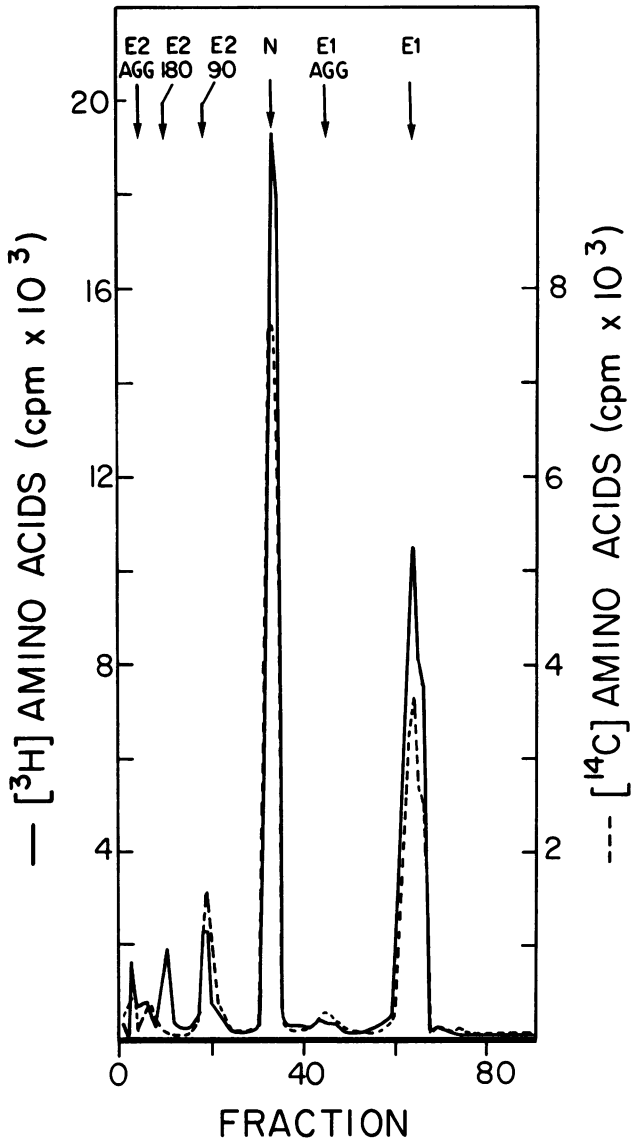

FIG. 3. Effect of trypsin on purified MHV-A59 virions. Virions labeled with ^{14}C-amino acids were purified by sucrose density gradient ultracentrifugation, treated with 100 μg/ml for 30 min at 37°C, and incubated with soybean trypsin inhibitor (200 μg/ml) for 1 h at 4°C. The virus was separated from trypsin and trypsin inhibitor by pelleting through a 20% sucrose cushion and then mixed with an equal amount of control virus labeled with ^{3}H-amino acids, which had been treated similarly except that trypsin had been omitted. The structural proteins of the trypsin-treated (---) and control (—) virus were analyzed by SDS-PAGE.

contained species which migrated with an apparent molecular weight of 90,000 while the two major peaks of methionine-labeled proteins contained N and El in order of elution (data not shown).

To clarify the relationship between the two 90K species, we took advantage of the finding that E2 is acylated (24). By analogy with other acylated viral envelope glycoproteins, the fatty acid is probably covalently bound to E2 near the lipid bilayer in the viral envelope. Therefore, we expected that only one of the two 90K cleavage products would be labeled with [^{3}H]palmitic acid. Virions doubly labeled with [^{3}H]palmitic acid and ^{14}C-amino acids were treated with trypsin, repurified, solubilized in 1% SDS, and analyzed by hydroxyapatite chromatography. Figure 5 shows the elution of four major peaks of amino acid-labeled proteins, two of which were associated with palmitic acid. Fractions from the first three peaks of these were analyzed by SDS-PAGE (Fig. 6). The first major peak eluted from the hydroxyapatite column contained 98% of the palmitic acid label in the virion and a 90K polypeptide (Fig. 6A). On SDS-PAGE, the palmitic acid label from this peak migrated with the tracking dye and was probably associated with lipids in the viral envelope. The 90K fragment in this peak did not contain covalently bound palmitic acid. The second major protein peak from the hydroxyapatite column contained the 50K nucleocapsid protein N, which was not labeled with palmitic acid (Fig. 6B). The third peak which eluted from hydroxyapatite consisted of two proteins, the 23K matrix-like glycoprotein E1 and a 90K species which contained comigrating and presumably covalently bound palmitic acid (Fig. 6C). Later fractions from the hydroxyapatite column (Fig. 5) consisted of monomeric and aggregated forms of E1 (data not shown). The 90K species with bound palmitic acid (Fig. 6C) was designated 90A, and the other 90K species which eluted first from the hydroxyapatite column and did not contain bound palmitic acid (Fig. 6A) was designated 90B. In experiments with virus which had not been trypsin treated, 90B could be isolated, and uncleaved 180K E2 eluted from hydroxyapatite with the 90A subunit (data not shown).

To demonstrate that the separation of 90A from 90B did not depend on the interaction of 90A with E1 or the association of 90B with non-covalently bound lipid, the two 90K species of E2 were analyzed on hydroxyapatite after the removal of the nucleocapsid, E1, and lipid. Trypsin-treated

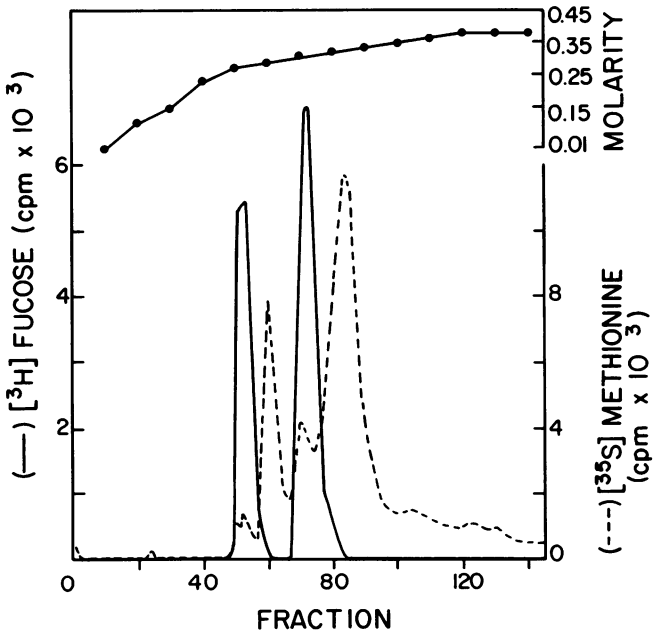

FIG. 4. Separation of MHV-A59 structural proteins by SDS-hydroxyapatite chromatography. Virions released from 17 Cl 1 cells grown in the presence of [^{3}H]fucose and [^{35}S]methionine were purified by sucrose density gradient ultracentrifugation, solubilized in 1% SDS in 0.01 M phosphate buffer (pH 6.5), and analyzed by hydroxyapatite chromatography in 0.1% SDS developed with a gradient of 0.15 M to 0.45 M phosphate buffer (pH 6.5).

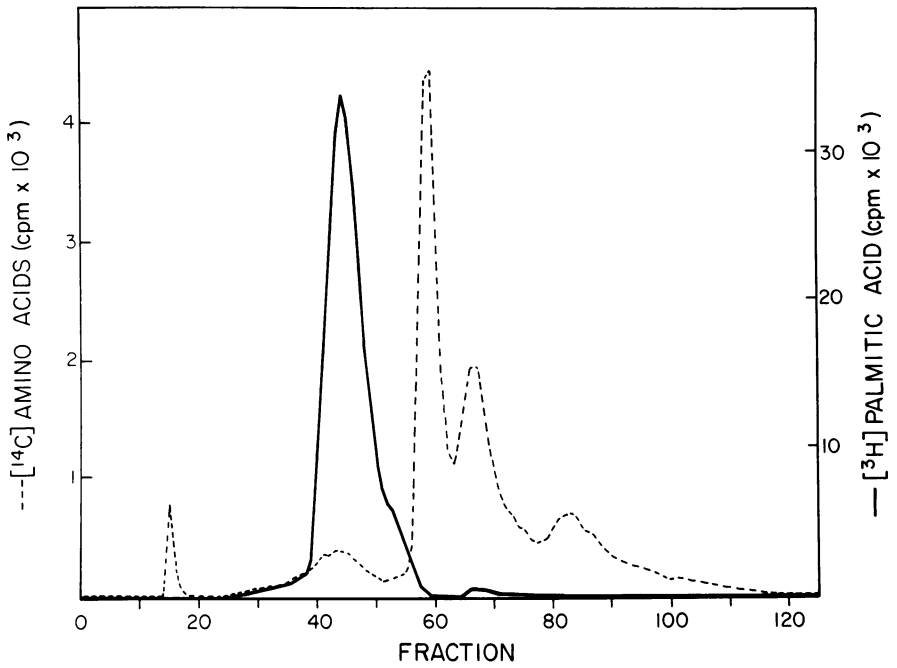

FIG. 5. Separation of the structural proteins of palmitic acid-labeled MHV-A59 by chromatography on hydroxyapatite in SDS. Purified virions labeled with [^{3}H]palmitic acid and ^{14}C-amino acids were treated with trypsin (100 μg/ml, 30 min, 37°C) and solubilized in 1% SDS, and viral proteins were analyzed by hydroxyapatite chromatography developed in a gradient containing 0.2 to 0.5 M sodium phosphate (pH 6.4), 0.2% SDS, and 1 mM dithiothreitol.

purified virions were solubilized with Nonidet P-40 (43), and 90A and 90B were separated from E1 and nucleocapsid by sucrose-Nonidet P-40 gradient ultracentrifugation and gel filtration on Fractogel TSK (HW-55S). The elution profiles of the two isolated 90K species (data not shown) were identical with those shown in Fig. 4 and 5. Though the mechanism of the separation of 90A and 90B on hydroxyapatite in SDS is unknown, this separation does not depend upon the association of either species with other components of the virion.

Limited digestion of 90A and 90B E2 by thermolysin and V8 protease. Separation of 90A and 90B E2 by hydroxyapatite chromatography in SDS permitted the comparison of products obtained from limited digestion with proteolytic enzymes. Thermolysin and *Staphylococcus aureus* V8 protease digests of mixtures of ^{3}H-amino-acid-labeled 90A and ^{14}C-amino-acid-labeled 90B are shown in Fig. 7. Different cleavage products were obtained from the two 90K species. At lower enzyme concentrations, 90B was readily cleaved into several fragments, whereas 90A was relatively resistant to proteolysis. At higher enzyme concentrations, degradation of 90A was apparent, and incubation with 2 μg of V8 protease produced a greater heterogeneity of 90A fragments than of 90B. These observations support the conclusion that the 90A and 90B subunits separated by hydroxyapatite chromatography represent different species with similar apparent molecular weights. Comparisons of the amino acid compositions of 90A and 90B (data not shown) also indicate that these two species have different primary sequences.

DISCUSSION

The isolation of two different 90K cleavage fragments from the 180K glycoprotein of MHV-A59 resolves the longstanding question of the relationship between the 90K and 180K forms of E2 (30, 35, 39, 40). It is now apparent that the identical tryptic peptides obtained from isolated 90K and 180K species was the result of an analysis of a mixture of two different 90K cleavage fragments (90A and 90B) which were compared with the tryptic peptides derived from the uncleaved 180K protein. Comparison of tryptic peptide maps of GP90 and GP84, the two large envelope glycoproteins of infectious bronchitis virus (IBV), with a 155K intracellular glycoprotein revealed that these two virion glycoproteins of IBV are also produced by the cleavage of a single precursor, GP155 (36). The E2 glycoprotein of IBV produced in primary chick embryo cells is cleaved asymmetrically and completely.

The size and number of the large glycoprotein species found on various coronaviruses varies. For example, with porcine transmissible gastroenteritis virus and canine coronavirus, Garwes and Reynolds (10) found only a 204K species. With IBV, however, Cavanagh (4) identified 84K and 90K species, and with porcine hemagglutinating encephalomyelitis virus, Pocock and Garwes (27) demonstrated 180K, 125K, and 100K species. An understanding of the proteolytic cleavage of the E2 glycoprotein may help explain why different coronaviruses appear to have one, two, or three high-molecular-weight species associated with the peplomers on the virion. If E2 is either completely cleaved, producing two comigrating products, or totally uncleaved, SDS-PAGE analysis may reveal only a single high-molecular-weight glycoprotein species. Identification of two high-molecular-weight species may reflect the presence of uncleaved E2 plus two comigrating cleavage products, such as 90A and 90B of MHV-A59, or just two separable cleavage products, such as the 94K and 84K species obtained with IBV (4). With MHV, acylation of E2 has proved to be a useful marker for distinguishing the two cleavage products when they are the same size. Coronaviruses with three high-molecular-weight glycoproteins may contain an

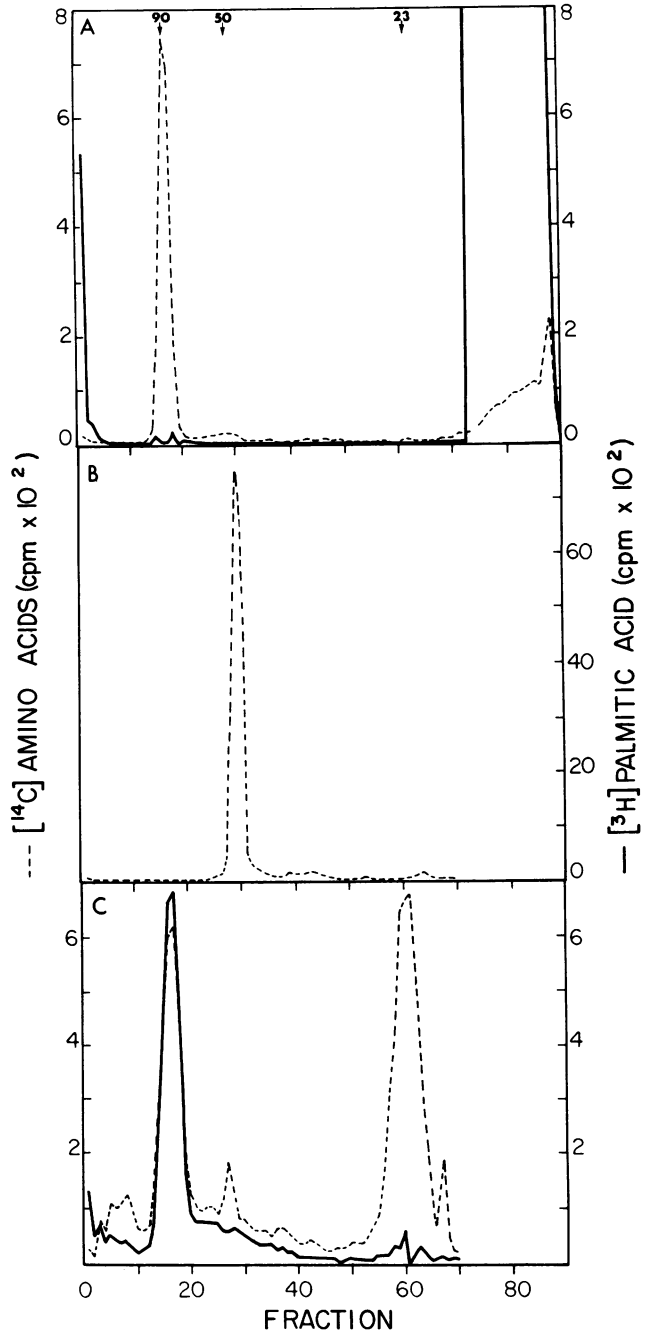

FIG. 6. Identification of viral proteins separated by hydroxyapatite chromatography. Fractions from the first three major peaks from the hydroxyapatite elution profile shown in Fig. 5 were analyzed by SDS-PAGE. (A) Fraction 18 contained 90K E2, and the palmitic acid migrated to the bottom of the gel. (B) Fraction 30 contained 50K N. (C) Fraction 18 contained 90K E2, and fraction 60 contained 23K E1.

uncleaved species as well as two cleavage products of different molecular weights. Further complexities may arise if one of the cleavage products is released from virions, as with some alphaviruses such as Sindbis virus (46). This occurs in the presence of reducing agent with porcine hemagglutinating encephalomyelitis virus (26) and IBV (5). In addition, bovine and porcine coronavirus strains may have an extra envelope glycoprotein, which may represent a different gene product (3, 15).

Studies with monospecific antibodies have shown that coronavirus-induced cell fusion is mediated by the large glycoprotein, E2, which both comprises the peplomers of the virion and is present on the plasma membrane of infected cells. Cell fusion by the JHM (6, 45) and A59 (K. V. Holmes, unpublished data) strains of MHV have been shown to be inhibited also by monoclonal antibodies to E2. The E2 on the cell surface is presumably activated by cellular proteases during the intracellular transport of the glycoprotein. However, nothing is known about the configuration of E2 on the plasma membrane of the cell.

We have shown that the activation of cell fusion activity of E2 on virions from 17 Cl 1 cells requires proteolytic cleavage. This has been demonstrated by the treatment of purified virions with trypsin. Trypsin treatment of coronavirus virions or infected cells has been observed by several investigators to have a number of effects that were probably related to the cleavage of the peplomeric glycoprotein. It is clear from the work of Storz and his co-workers (37) with bovine coronaviruses that proteolytic activation of some coronaviruses may be required for in vitro cultivation of the virus and for plaque formation. Similar findings were obtained by Yoshikura and Tejima (48) with some strains of MHV and by Otsuki and Tsubokura with several strains of IBV (25) which did not form plaques unless trypsin was added to the overlay medium. The accompanying paper shows that cleavage of MHV-A59 is host cell dependent. In addition, concentrated virions from Sac$^-$ cells, which have

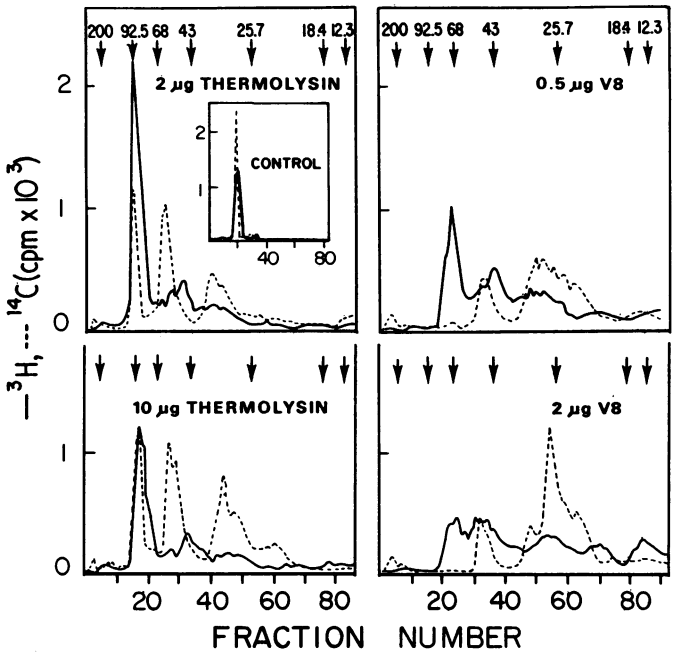

FIG. 7. Comparison of peptides of 90A and 90B released by limited digestion with thermolysin and V8 protease. Mixtures containing approximately equal counts per minute of ^{3}H-amino-acid-labeled 90A and ^{14}C-amino-acid-labeled 90B were incubated for 1 h at 37°C with thermolysin (2 or 10 μg) or with V8 protease (0.5 or 2 μg) and analyzed by SDS-PAGE. Inset shows that purified ^{3}H-amino-acid-labeled 90A and ^{14}C-amino-acid-labeled 90B comigrate at 90K before enzyme digestion.

100% cleaved E2, can cause the rapid fusion of L2 cells (8) without requiring trypsin activation as does virus from 17 Cl 1 cells. These results suggest that host-dependent proteolytic activation of virus-induced cell fusion may play an important role in coronavirus spread and pathogenesis. Activation of the hemagglutinin of IBV was shown to require treatment of virions with trypsin (7). However, in subsequent studies, the response of IBV to trypsin was shown to be strain dependent (1), and in some cases trypsin inactivated or destroyed the hemagglutinating activity of IBV (1) and other hemagglutinating coronaviruses such as the human coronavirus OC38/43 (14). Reexamination of the effects of trypsin on E2 in coronavirus virions may clarify the variation in response of the hemagglutinin of various coronaviruses to trypsin.

While it is clear that cleavage of MHV-E2 is a host-dependent function (8), it has not been possible to determine whether cleavage of the E2 glycoprotein is required for coronavirus infectivity, as there is as yet no source of MHV-A59 with entirely uncleaved E2. Although virions with completely uncleaved E2 may be noninfectious, partial cleavage of E2 as by 17 Cl 1 cells is sufficient to activate coronavirus infectivity but not rapid cell fusion. Further cleavage of E2 by trypsin does not enhance MHV infectivity but does activate rapid fusion. Similarly, partially cleaved influenza hemagglutinin glycoprotein is sufficient to activate infectivity (17). Possibly, a higher local concentration of cleaved glycoprotein is required for rapid fusion than for infectivity.

The similarity in proteolytic activation of cell-fusing activity of coronaviruses, positive-strand RNA viruses, and negative-strand orthomyxo- and paramyxoviruses (13, 19, 20, 32, 47) is of considerable interest, as this host-controlled modification is an important determinant of virus pathogenicity (2, 9, 33). The amino acid sequence at the new amino terminus generated by the cleavage of fusion proteins of orthomyxo- and paramyxoviruses is critical to cell-fusing activity, as synthetic peptides with the same sequence have been shown to competitively inhibit the biological activities of the native cleaved proteins (28, 29). Studies are in progress to determine whether the new terminal domains of 90A and 90B created by trypsin cleavage of E2 resemble analogous domains of the fusion glycoproteins of orthomyxo- and paramyxoviruses and whether the trypsin cleavage site is the same as the cleavage site of Sac^- cellular protease, which can also activate cell-fusing capacity of MHV-E2 (8). Knowledge of the amino acid sequences adjacent to the trypsin and host cell protease cleavage sites will help in elucidating the mechanisms of coronavirus fusion and permit comparison with fusion by other virus groups.

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