

Characterization of a Coronavirus¹

II. Glycoproteins of the Viral Envelope: Tryptic Peptide Analysis

LAWRENCE S. STURMAN² AND KATHRYN V. HOLMES³

*Division of Laboratories and Research, New York State Department of Health, Albany, New York 12201 and
Department of Microbiology, University of Texas, Southwestern Medical School, Dallas, Texas 75235*

Accepted November 29, 1976

Two species of membrane-associated glycoproteins have been identified in the coronavirus virion. They are readily distinguished on the basis of size, radiolabeling characteristics, and location in relation to the lipid bilayer. The larger glycoprotein is highly labeled by both radiolabeled fucose and glucosamine. This species is found in two forms, GP180 and GP90, with apparent molecular weights of 180,000 and 90,000. GP180 can be converted to GP90 *in vitro* by treatment of virions with trypsin. Analysis of tryptic digests of GP90 and GP180 give identical peptide patterns. Based on pronase and bromelain sensitivities, GP180/90 is the only protein which is located entirely external to the viral envelope. It appears to comprise the characteristic long, petal-shaped peplomers of the virion. The smaller glycoprotein, GP23, has an apparent molecular weight of 23,000 and is labeled by radiolabeled glucosamine but not by fucose. The level of glucosamine-labeling of GP23 is about 1/10 that of GP180/90. GP23 appears to possess two distinct domains: a smaller, carbohydrate containing region which is found outside the viral envelope, and a larger portion, highly labeled by methionine, which is integrally associated with the viral membrane. A new nomenclature is proposed for the three major coronavirus structural proteins. The two envelope glycoproteins, GP23 and GP180/90 are designated E1 and E2, respectively; the inner core protein, VP50, is designated N.

INTRODUCTION

Coronaviruses have a distinctive appearance (McIntosh, 1974). In negatively stained preparations the external surface of the virion envelope is covered with peplomers, 20 nm long, which are widely spaced and irregularly shaped, broader peripherally than at the base. This produces the appearance of a corona surrounding the virion from which the descriptive name coronavirus is derived.

¹ A portion of this work was presented at the 74th Annual Meeting of the American Society for Microbiology (Abstracts, p. 219, 1974).

² Address reprint requests to Dr. Lawrence Sturman, Division of Laboratories and Research, New York State Department of Health, Albany, New York 12201.

³ Present address: Department of Pathology, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20014.

The structural proteins of the coronavirus A59 virion have been identified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Sturman, 1977). Under standard conditions, six major polypeptide species are found with apparent molecular weights in the range from 180,000 to 23,000. Several of these species have been shown to be different forms of the same molecule. If the viral proteins are solubilized in SDS at 25° or 37°, only four size classes of polypeptide species are seen: GP23, VP50, GP90, and GP180. The use of boiling in sample preparation causes the appearance of two or more additional species accompanied by a proportionate decrease in GP23. The electrophoretic mobility of GP23 is reduced further by boiling in the presence of β -mercaptoethanol (β -MSH) or dithiothreitol (DTT) (Sturman, 1977).

Based on susceptibility to proteases in the intact virus, GP90, GP180, and GP23 are envelope proteins. GP90 and GP180 are completely accessible to pronase and bromelain and appear to reside outside the viral membrane (Sturman, 1977). However, only about 20% of the GP23 molecule is digested under the same conditions. The major portion of GP23 is presumed to be within the viral envelope (Sturman, 1977). The surface projections of enveloped viruses have been shown to be glycoproteins (reviewed by Lenard and Compans, 1974). In the present study the membrane-associated coronavirus glycoproteins are characterized. Based on tryptic peptide analysis and cleavage by trypsin *in vitro*, GP90 and GP180 are shown to be the same. The identity, radiolabeling characteristics, and location of the two coronavirus glycoproteins, GP180/90 and GP23, are the subject of this communication.

MATERIALS AND METHODS

Polyacrylamide Gel Electrophoresis

The procedures employed for the high pH-discontinuous buffer SDS-PAGE have been described previously (Sturman, 1977). The conditions used for preparation of the sample are given in the text.

Tryptic Peptide Analysis

[³⁵S]Methionine-labeled viral proteins were first separated by PAGE without boiling or addition of reducing agents and then recovered from crushed SDS-polyacrylamide gels in 0.05 M ammonium bicarbonate and 0.05% SDS by freezing and thawing twice and incubation at 37° for 16 hr. The residual gel was removed by filtration through a 0.45- μ m Swinnex filter unit (Millipore Corp.). Most of the SDS was precipitated with 1 M KCl. The separated viral proteins were dialyzed against 1% ammonium bicarbonate plus 0.13% Triton X-100 for several days at 4° with daily changes of buffer and then lyophilized twice. Each species, resuspended in 1% ammonium bicarbonate was treated with 50 μ g of trypsin for 16 hr at 37°. The tryptic peptides were lyophilized twice and resublimed in 10% acetic acid at 25°. Ten micro-

liters of each mixture containing 50,000–100,000 cpm was placed on a 20 \times 20-cm thin-layer cellulose plate (EM-reagent, Avicell microcrystalline, Brinkmann Instruments, Inc., Westbury, N.Y.), and two-dimensional chromatography was performed as described by Bellisario *et al.* (1973). The first solvent system contained *n*-butanol/acetic acid/water (200/30/75, v/v/v), and the second separation was performed at right angles in *n*-butanol/pyridine/acetic acid/water (15/10/3/12, v/v/v). The sheets were developed by exposure for 6–8 hr in each dimension and dried overnight after each separation. The methionine-containing polypeptides were detected by autoradiography with Kodak Royal Blue X-Omat X-ray film.

Electron Microscopy

Coronavirus virions concentrated and purified by polyethylene glycol precipitation and sucrose density gradient sedimentation (Sturman and Holmes, in preparation) were incubated with enzymes, fixed with 1% glutaraldehyde in TMEN buffer, pH 6, (Sturman, 1977) for 5 min, and negatively stained with 2% phosphotungstic acid or sodium silicotungstate on carbon-coated Formvar grids. Random fields of the grids were examined with a Philips 301 electron microscope at 60 kV.

Chemicals and Isotopes

Most of the chemicals which were employed have been described previously (Sturman, 1977). In addition, 1-butanol and glacial acetic acid (both reagent grade) were purchased from J. T. Baker Chemical Co., Phillipsburg, N.J. Pyridine (Spectroquality) was obtained from Matheson, Coleman & Bell, Norwood, Ohio and trypsin (Type XI), DCC treated, dialyzed, salt-free, and lyophilized, from Sigma Chemical Co., Saint Louis, Mo. Lima bean trypsin inhibitor was purchased from Worthington Biochemical Corp., Freehold, N.J. A purified fraction of bromelain prepared by the method of Scoocca and Lee (1969) was kindly supplied by Dr. A. Tarentino. No glycosidase activity was detected in this preparation with

p-nitrophenol glycosides of β -*n*-acetylglucosamine, α - and β -mannose, α - and β -galactose, and α -fucose.

L-[6-³H]Fucose, 13.4 Ci/mmol, D-[1-¹⁴C]glucosamine hydrochloride, 56.6 mCi/mmol, and D-[6-³H] (N)glucosamine hydrochloride, 7.3 Ci/mmol, were obtained from New England Nuclear Corp., Boston, Mass. D-[1-¹⁴C]Glucosamine hydrochloride, 25 mCi/mmol, was also purchased from Amersham/Searle Corp., Arlington Heights, Ill. The labeled amino acids which were employed have been described earlier (Sturman, 1977).

RESULTS

Identification of Viral Glycoproteins

For the identification of coronavirus glycoproteins, A59 virus was grown in the presence of radiolabeled glucosamine and fucose. Glucosamine and fucose were selected because both of these labeled compounds have been shown to be incorporated into viral glycoproteins mostly unchanged (Strauss *et al.*, 1970 and Klenk *et al.*, 1970). Viral polypeptides were analyzed by high pH-discontinuous buffer SDS-PAGE. The procedure described by Laemmli was first employed; samples were heated at 100° for 2 min in 2% SDS with 5% β -MSH before being placed on the gel (Laemmli, 1970). The electropherogram in Fig. 1A of A59 polypeptides doubly labeled with amino acids and glucosamine shows that five of the six polypeptide species detected by this method were labeled with glucosamine. Based upon their apparent molecular weights, these polypeptides have been designated GP23 (GP indicates that the polypeptide species was labeled by glucosamine), GP38, VP50, GP60, GP90, and GP180 (Sturman, 1977). Only VP50 was found to be unlabeled by glucosamine. Among the other species, two levels of glucosamine labeling are apparent. The ratio of glucosamine:amino acid-label in GP90 and GP180 is approximately tenfold greater than in GP23, GP38, and GP60. The pattern of labeling with fucose showed a corresponding but slightly different result. GP90 and GP180 were highly labeled with fucose, whereas GP23, GP38, and GP60 contained no de-

tectable fucose label (Fig. 1B). A substantial amount of fucose and glucosamine label was found also at the origin. This will be considered further below. The lack of fucose label in the smaller glycoprotein species was a consistent finding with A59 virus produced in all four cell types examined: AL/N, Py-AL/N, Balb-3T3, and 17 Cl 1 (unpublished data).

It has been reported that radiolabeled glucosamine may bind to some proteins nonenzymatically, giving rise to erroneous labeling results (Angello and Hauschka, 1974). Since glucosamine label was present only in low levels in GP23, GP38, and GP60, the possibility of noncovalent association between glucosamine and coronavirus polypeptides was examined. A clarified suspension of crude, freshly prepared released virus, labeled with [¹⁴C]amino acids was incubated with [³H]glucosamine, 2 μ Ci/ml, for an additional 18 hr under the same conditions as are employed for virus production, but without cells. The virus was then purified and analyzed by SDS-PAGE. No glucosamine label was detected associated with any polypeptide in the gel (data not shown). In comparison, virus grown in the presence of [¹⁴C]amino acids and [³H]glucosamine contained $3\text{--}4.5 \times 10^3$ cpm of [³H]glucosamine in peak fractions of GP38 and GP23. This result is consistent with the conclusion that the glucosamine label found in these species represents covalently bound labeled carbohydrate.

Effect of Conditions of Preparation on the Number of Glycoproteins which are Labeled with Glucosamine but not Fucose

The preparative conditions for SDS-PAGE markedly affect the migration of some coronavirus protein in polyacrylamide gels (Sturman, 1977). SDS-PAGE of A59 virus dissociated in SDS at 100° contained species with apparent molecular weights of 38,000 (GP38) and 60,000 (GP60). It has been shown that these species were not found in similar gels with samples prepared at 25° or 37° (Sturman, 1977). It was also found that β -MSH or DTT in the heated mixture brought about

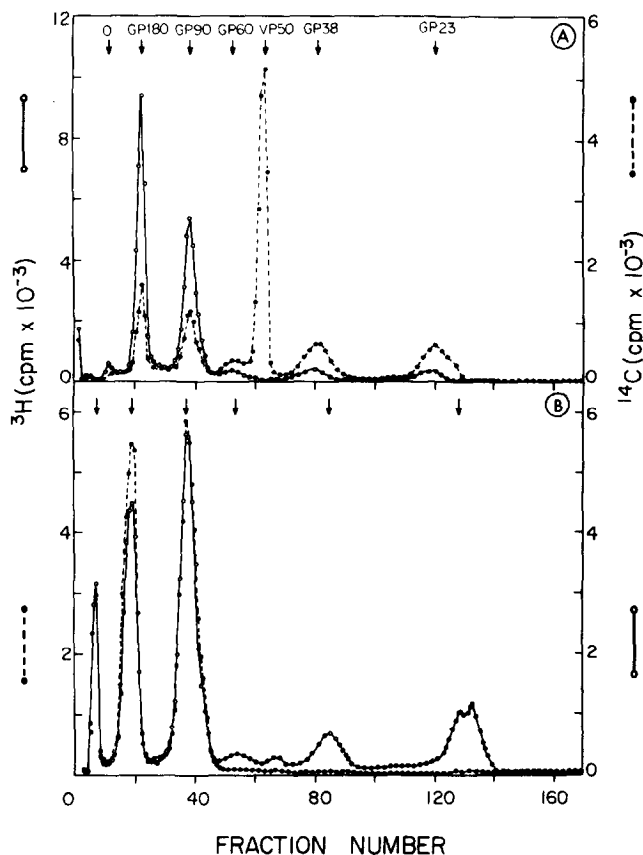


FIG. 1. SDS-PAGE of the polypeptides of the coronavirus A59 virion labeled with amino acids, glucosamine, and fucose. The virus was grown for 26 hr in the presence of 1 μ Ci/ml of [14 C]amino acids and 2 μ Ci/ml of [3 H]glucosamine, or 0.8 μ Ci/ml of [14 C]glucosamine and 4 μ Ci/ml of [3 H]fucose. The purified virus containing 5% β -MSH was heated to 100° for 1.5 min before being placed on the gel, and arrows indicate the position of the peak fractions of the virion polypeptides. In this and the following figures, migration is from left to right. (A) [14 C]amino acids (●--●) and [3 H]glucosamine (○—○); (B) [14 C]glucosamine (○—○) and [3 H]fucose (●--●).

an increase in the proportion of slower migrating species. The effect of preparation at 25° in the absence of reducing agent on the migration of glucosamine- and fucose-labeled A59 polypeptides in SDS-polyacrylamide gels is shown in Fig. 2. Under these conditions, GP23 was the only species found which was labeled with glucosamine and not with fucose.

A substantial amount of fucose and glucosamine labeled material is present also at the top of the resolving gel. Aggregation of some viral glycoproteins is especially pronounced whenever a sample is applied without boiling. From a comparison of the ratios of glucosamine, fucose, and valine labels in these aggregates and in the indi-

vidual proteins, it is apparent that the aggregates consist primarily of GP90 and GP180 (Figs. 2, 3A).

Protease Digestion of the Glucosamine-Labeled Portion of GP23

The coronavirus glycoproteins GP23, GP90, and GP180 are degraded by treatment of the intact virus with proteases such as pronase and bromelain. However, only about 20% of the GP23 molecule is susceptible under these conditions. To account for this result it has been postulated that GP23 is composed of two domains, a smaller region extrinsic to the viral envelope and a larger portion which is intramembranous and perhaps spans the viral

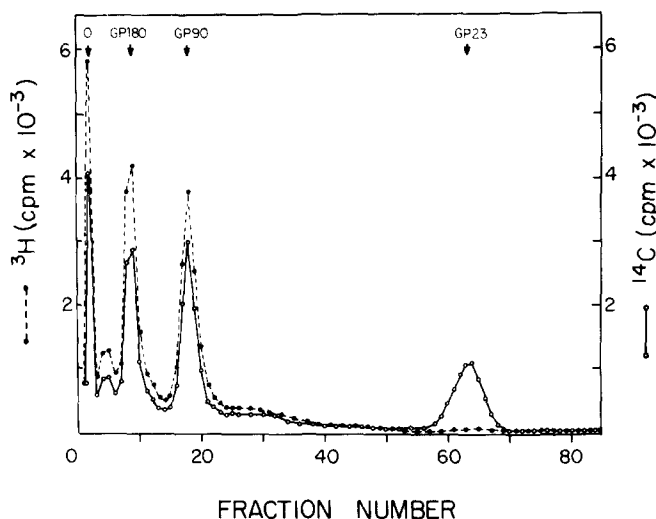


FIG. 2. SDS-PAGE of the polypeptides of coronavirus A59 labeled for 24 hr with 3 $\mu\text{Ci/ml}$ of [^3H]fucose and 2 $\mu\text{Ci/ml}$ of [^{14}C]glucosamine. Virus was suspended in 0.060 M Tris-phosphate buffer (pH 7.0) containing 2% SDS, 10% glycerol, and 0.001% BPB at 25°, no β -MSH (or DTT).

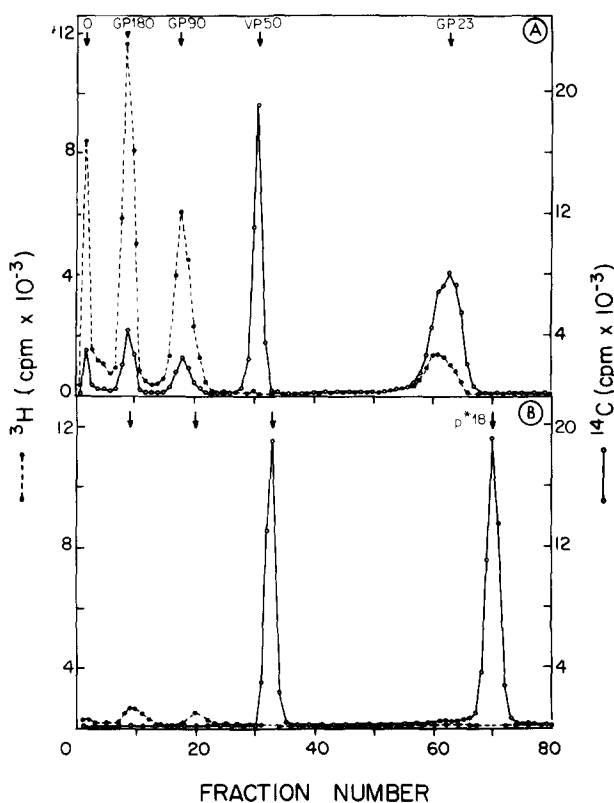


FIG. 3. Effect of bromelain on coronavirus A59 virion polypeptides labeled with [^3H]glucosamine and [^{14}C]valine. Virus was grown for 24 hr in the presence of 2 $\mu\text{Ci/ml}$ of [^3H]glucosamine and 1 $\mu\text{Ci/ml}$ of [^{14}C]valine. One portion of purified virus was incubated at 37° for 2.75 hr with 1.0 mg/ml of bromelain and 0.1 mM β -MSH, while the other served as a control. Bromelain treatment was terminated by addition of PCMB, 1 mM final concentration. Virus was pelleted and resuspended in 0.060 M Tris-phosphate (pH 6.7), 2% SDS, 10% glycerol, and 0.001% BPB at 25°, and the virus was analyzed without heating and additional β -MSH. (A) control; (B) bromelain treated. [^3H]glucosamine (●---●), [^{14}C]valine (○—○).

membrane (Sturman, 1977). In order to determine which region of GP23 contains the glucosamine label, it was proposed to analyze the PAGE pattern of carbohydrate-labeled bromelain-treated virus. However, commercially available bromelain (Sigma, grade II) is a crude preparation which contains many glycosidase activities including β -N-acetylglucosaminidase, α - and β -mannosidase, α - and β -galactosidase, and α -fucosidase (A. Tarentino, personal communication). Therefore, a purified protease fraction from bromelain which possessed none of these glycosidase activities was used. SDS-PAGE of the polypeptides from bromelain-treated A49 virus doubly labeled with [3 H]glucosamine and [14 C]amino acids revealed that the bromelain-resistant portion of GP23 (designated p*18) did not contain glucosamine (Fig. 3B). Therefore, the labeled carbohydrate appears to be located on the segment of GP23 which lies outside the viral envelope. This carbohydrate-labeled portion of GP23 is not essential for the production of those species of lower mobility (such as GP38 and GP60) which arise after heating at 100° and in the presence of β -MSH or DTT, since p*18 also gives rise to similar anomalous forms (p*31 and p*48) under the same conditions (Sturman, 1977).

Tryptic Peptide Analysis of Coronavirus Proteins

GP90 and GP180 exhibited the same relative patterns of labeling with several amino acids and carbohydrates (Sturman, 1977 and above). The tryptic peptides of [35 S]methionine-labeled coronavirus proteins were analyzed to determine whether there is a relationship between these two or between any of the other species. Individual viral proteins were recovered from crushed SDS-polyacrylamide gels. The [35 S]methionine-labeled tryptic peptides produced were separated by two-dimensional thin-layer chromatography and detected by autoradiography. The results are shown in Fig. 4. The peptide patterns obtained from GP90 and GP180 are nearly identical. The tryptic peptide patterns from GP23 and VP50 are distinctive and

different from GP90 and GP180. Thus the coronavirus virion contains two distinct glycoprotein species, GP23 and GP180/90.

Conversion of GP180 to GP90 by Trypsin

GP180 could be converted to GP90 by treatment of the virions with trypsin *in vitro*. As shown in Fig. 5, incubation of A59 virions with 10 μ g/ml or 1 mg/ml of trypsin for 50 and 150 min, respectively, at 37° resulted in the loss of valine and glucosamine labels in GP180 and a comparable increase in both labels in GP90. The amounts of valine and glucosamine labels found in each of the virion polypeptides before and after trypsin treatment shown in Fig. 5 are given in Table 1. The data are expressed as ratios in this table so that the proportion of each label in the same polypeptide species in different gels can be compared. VP50 was used as the internal standard for [3 H]valine and GP23 for [14 C]glucosamine. The sum of the [3 H]valine in GP90 and GP180 before trypsin treatment equaled 35% of that in VP50. After trypsin treatment, which produced an approximately 90% reduction in label in GP180, the amount of [3 H]valine in GP90 alone increased from 20% to 32–36% of that of VP50 (Table 1, Fig. 5). Similarly the amount of [14 C]glucosamine label found in GP90 after trypsin treatment was equal to 88–96% of the sum of the glucosamine label found in GP90 plus GP180 before trypsin treatment (Table 1, Fig. 5). Thus GP90 appears to be quantitatively derived from GP180 by treatment of virions with trypsin *in vitro*. Virus infectivity was only slightly affected by trypsin treatment. Depending on the concentration of trypsin and condition employed, infectivity was changed by ± 0.3 log. None of the other structural polypeptides was affected by trypsin, and after isopycnic sedimentation of trypsin-treated virus in a sucrose gradient (unpublished data) less than 1% of the amino acid label had been released from the virion.

Electron microscopic examination of virions after conversion of GP180 to GP90 by trypsin revealed no structural alteration of the surface projections of the virion (Fig. 6a, b). In contrast to the lack of morpholog-

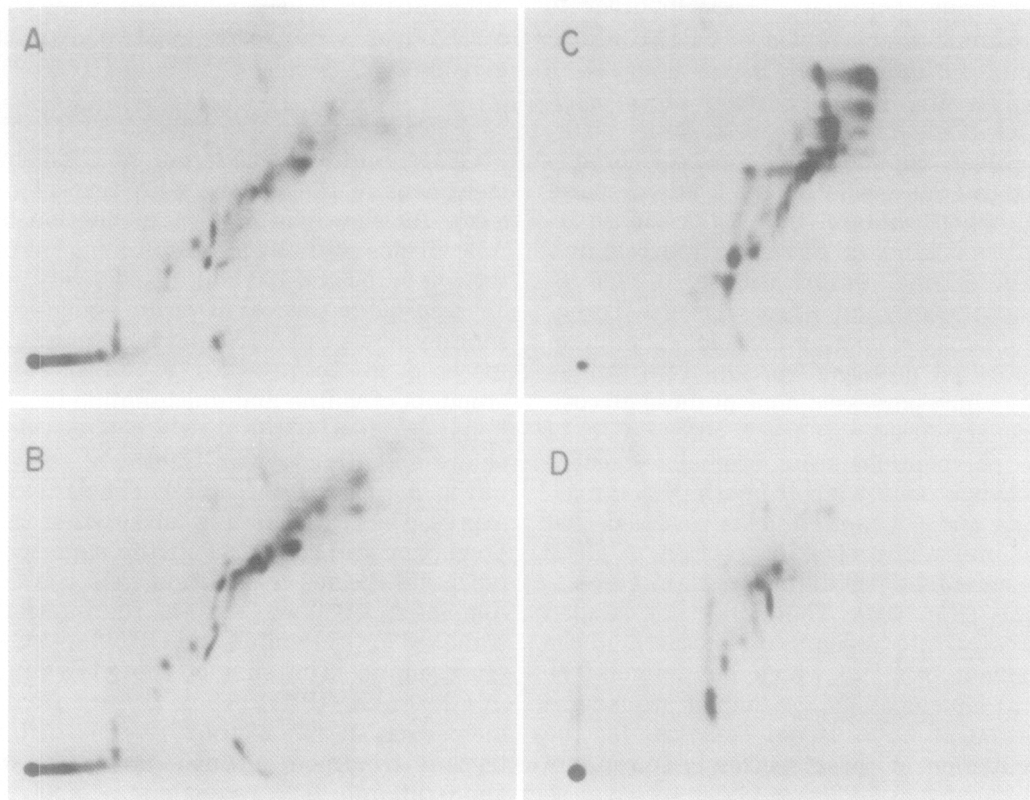


FIG. 4. Autoradiograms of the [^{35}S]methionine-labeled tryptic peptides from the structural proteins of coronavirus A59: GP180, GP190, VP50, and GP23, separated by two-dimensional chromatography on thin-layer cellulose plates. (A) GP180, (B) GP90, (C) VP50, and (D) GP23.

ical change following trypsin treatment, pronase and bromelain produced complete removal of the surface projections from the virions (Figs. 6c, d). Previous studies (Sturman, 1977 and see 3B above) have shown that treatment of A59 virus by these proteases caused the loss of GP180 and GP90, as well as partial degradation of GP23 and a reduction in virus infectivity by 4.5 log.

DISCUSSION

Two membrane-associated glycoproteins have been identified in the coronavirus A59 virion. They are readily distinguished on the basis of size, radiolabeling characteristics, and location in relation to the lipid bilayer:

(1) The larger glycoprotein is labeled by both radiolabeled fucose and glucosamine. This species is found in two forms with apparent molecular weights of approxi-

mately 180,000 (GP180) and 90,000 (GP90). Two dimensional chromatographic analysis of tryptic digests of GP180 and GP90 reveals identical peptide patterns. GP180 can be converted to GP90 *in vitro* by treatment of virions with trypsin. Based on pronase and bromelain sensitivities, this glycoprotein is the only viral protein which is located entirely external to the viral envelope.

(2) The smaller coronavirus glycoprotein, GP23, has an apparent molecular weight of 23,000 and is labeled by radiolabeled glucosamine, but not by fucose. The level of glucosamine labeling of GP23 is about 1/10 that of the larger glycoprotein. Treatment of coronavirus virions with proteolytic enzymes such as pronase or bromelain causes complete removal of the surface projections from the virion and loss of GP90 and GP180. However, such treatment produces only a 20% reduction in the

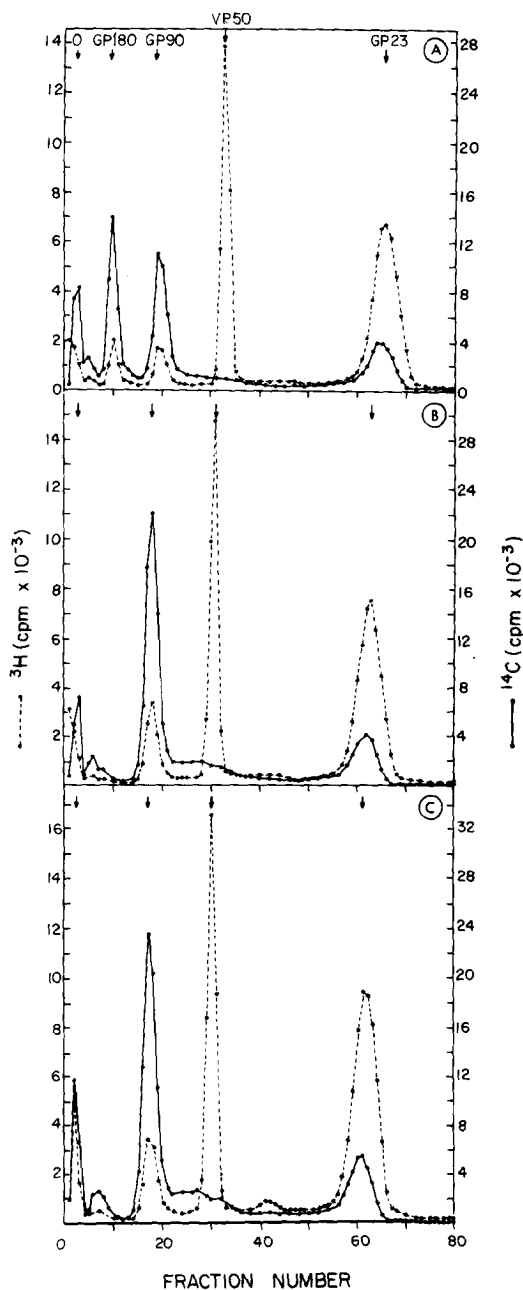


FIG. 5. Conversion of GP180 to GP90 by trypsin treatment of virions. SDS-PAGE of coronavirus A59 labeled with [^3H]valine and [^{14}C]glucosamine. Virus was grown for 24 hr in the presence of $4\ \mu\text{Ci/ml}$ of [^3H]valine and $2\ \mu\text{Ci/ml}$ of [^{14}C]glucosamine. The virus was purified and treated as follows: one portion was kept at 4° without trypsin, a second portion was incubated with $10\ \mu\text{g/ml}$ of trypsin for 50 min at 37° , and the third portion was incubated with $1\ \text{mg/ml}$ of trypsin for 150 min at 37° . The reaction with trypsin was terminated by addition of a threefold

apparent size of the GP23 molecule. The segment which is degraded includes the region to which the glucosamine label is bound. Although the remainder of GP23, designated p*18, is inaccessible to proteolytic enzymes in the intact virion, it is sensitive to proteolysis upon disruption of the viral envelope in 0.1% SDS. Thus GP23 appears to possess two distinct domains: a smaller, carbohydrate-containing region which is found outside the viral envelope and a larger portion which is integrally associated with the viral membrane. Since only a small proportion of the GP23 molecule lies outside of the viral envelope, many of the potentially suitable amino acid residues of this species may be inaccessible for glycosylation. This may account, at least in part, for the lower level of glucosamine label in GP23 compared with GP180/90. However, the absence of fucose label in GP23 suggests that there are also compositional differences in the carbohydrate units associated with the smaller and larger glycoproteins.

GP23 has been shown to be much more highly labeled by [^{35}S]methionine than any of the other virion polypeptides (Sturman, 1977). The significance of this finding is not known. Methionine is a nonpolar amino acid (Tanford, 1962) with a high potential for regions in proteins which contain β -pleated sheet conformations (Chou and Fasman, 1974).

Since the external portion of GP23 is quite small, the characteristic long, petal-shaped peplomers of the coronavirus virion are probably composed largely of GP180 and/or GP90 in monomeric or multimeric form. By analogy with some myxovirus and paramyxovirus glycoproteins (Lazarowitz *et al.*, 1971; Klenk *et al.*, 1972; Skehel, 1972; Scheid and Choppin, 1974), GP90 presumably arises through cleavage of GP180. As in those cases, the precursor can be converted to the product, *in vitro*,

excess of lima bean trypsin inhibitor. The virus was pelleted and resuspended at 25° in $0.060\ \text{M}$ Tris-phosphate, 2% SDS, 10% glycerol, and 0.001% BPB. (A) control, (B) $10\ \mu\text{g/ml}$ of trypsin, (C) $1\ \text{mg/ml}$ of trypsin. [^3H]valine (\bullet — \bullet), [^{14}C]glucosamine (\circ — \circ).

TABLE 1

RATIOS OF INCORPORATION OF [³H]VALINE AND [¹⁴C]GLUCOSAMINE INTO UNTREATED AND TRYPSIN-TREATED VIRION POLYPEPTIDES

Polypeptide species (X)	Relative incorporation of [³ H]valine ^a			Relative incorporation of [¹⁴ C]glucosamine ^b		
	0 ^c	10 µg/ml	1 mg/ml	0 ^c	10 µg/ml	1 mg/ml
GP180	15	2	2	131	10	8
GP90	20	36	32	155	274	253
GP23	146	148	151	100	100	100

^a $\frac{(X)}{VP50} \cdot 10^2$.

^b $\frac{(X)}{GP23} \cdot 10^2$.

^c Amount of trypsin.

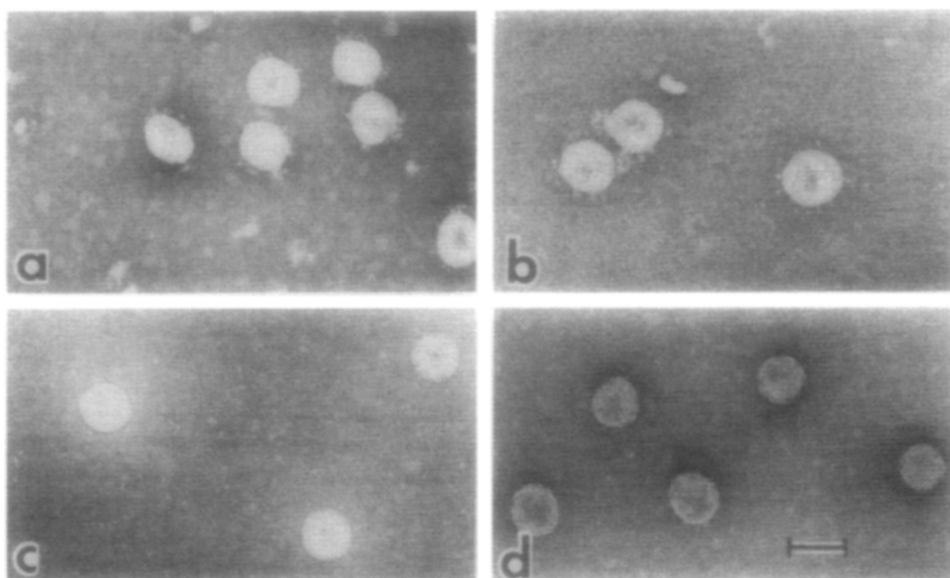


FIG. 6. Electron microscopic appearance of A59 virions after treatment with trypsin, pronase, or bromelain. (a) control virions; (b) trypsin treated, 1 mg/ml, 150 min; (c) pronase treated, 1 mg/ml 180 min; (d) bromelain treated, 1 mg/ml, 180 min. Virions were incubated with enzymes at 37°, fixed with glutaraldehyde, and negatively stained. $\times 75,000$. Bar, 100 nm.

by incubation of the virus with trypsin. *In vitro* cleavage of GP180 is associated with only slight (twofold) changes in infectivity. This is in contrast with the results obtained from trypsin treatment of several strains of influenza and Sendai viruses in which marked increases (up to 100-fold) in infectivity were found (Klenk *et al.*, 1975; Lazarowitz and Choppin, 1975; Scheid and Choppin, 1974; Homma and Ohuchi, 1973).

The enzyme(s) responsible for cleavage of GP180 *in vivo* has not been identified. The relative proportions of GP180 and

GP90 in the virions were not affected by production of the virus in the presence of tosyl-L-lysyl-chloromethane (TCLK) or tosyl-L-phenylalanyl-chloromethane (TPCK) (both 10^{-4} M), inhibitors of trypsin- and chymotrypsin-like enzyme activities, or in media lacking fetal bovine serum, which is essential for plasmin-mediated cleavage of myxovirus glycoproteins (unpublished data).

Another possibility which should be considered is that GP180 is simply a dimer of GP90. In that case, aggregation must be

mediated through a trypsin-sensitive linker.

When SDS-PAGE is performed on a sample which is applied without boiling and in the absence of any reducing agent, a substantial amount of A59 virus-specific polypeptide remains unresolved at the origin of the separating gels (Figs. 1-3 and 5 above, and Sturman, 1977). Based on the ratios of carbohydrate and amino acid labels, this form represents aggregates of GP180/90 which have either formed or failed to dissociate during preparation of the sample or during migration through the spacer gel. Treatment of intact virions with bromelain, which results in the removal of GP90 and GP180, also eliminates the appearance of such aggregates (Fig. 3B). The significance of aggregation of GP180/90 in the intact virion will be considered in another report (Sturman and Holmes, manuscript in preparation).

Based upon the results described in this and in the preceding paper (Sturman, 1977), the following designations are proposed for the three major coronavirus structural proteins: the two envelope glycoproteins, GP23 and GP180/90 are designated E1 and E2, respectively, and the inner core protein, VP50, is designated N (Table 2).

Three antigens have been reported to be associated with the coronavirus virion (Tevethia and Cunningham, 1968; Mengeling, 1972; Hierholzer *et al.*, 1972; and Bohac and Derbyshire, 1975). The relationship between these antigens and the three virion structural proteins which have been identified is not known. The antigenic roles of E1, E2, and N are currently being investigated.

ACKNOWLEDGMENTS

We gratefully acknowledge the excellent technical assistance of Mrs. Gale Schmidt, Mr. Peter Grob,

Mr. Gustave Boesch, and Ms. Rae Allen. The authors also wish to thank Dr. Ronald Bellisario for his advice concerning the tryptic peptide analyses and Dr. Anthony Tarentino for helpful discussions.

This work was supported in part by Grant No. CA 17713 from the National Cancer Institute, DHEW.

REFERENCES

- ANGELO, J. C., and HAUSCHKA, S. D. (1974). Glucosamine binding to seven proteins: Its possible relevance to cell surface and conditioned medium studies. *Biochim. Biophys. Acta* 367, 148-164.
- BELLISARIO, R., CARLSEN, R. B., and BAHL, O. P. (1973). Human chorionic gonadotropin. Linear amino acid sequence of the α subunit. *J. Biol. Chem.* 248, 6796-6809.
- BOHAC, J., and DERBYSHIRE, J. B. (1975). The demonstration of transmissible gastroenteritis viral antigens by immunoelectrophoresis and counter-immunoelectrophoresis. *Canad. J. Microbiol.* 21, 750-753.
- CHOU, R. Y., and FASMAN, G. D. (1974). Prediction of protein conformation. *Biochemistry* 13, 222-245.
- HIERHOLZER, J. C., PALMER, E. L., WHITFIELD, S. G., KAYE, H. S., and DOWDLE, W. R. (1972). Protein composition of coronavirus OC43. *Virology* 48, 516-527.
- HOMMA, M., and OHUCHI, M. (1973). Trypsin action on the growth of Sendai virus in tissue culture cells. III. Structural differences of Sendai viruses grown in eggs and tissue culture cells. *J. Virol.* 12, 1457-1465.
- KLENK, H. D., CALIGUIRI, L. A., and CHOPPIN, P. W. (1970). The proteins of the parainfluenza virus SV5. II. The carbohydrate content and glycoproteins of the virion. *Virology* 42, 473-481.
- KLENK, H. D., SCHOLTISSEK, C., and ROTT, R. (1972). Inhibition of glycoprotein biosynthesis of influenza virus by D-glucosamine and 2-deoxy-D-glucose. *Virology* 49, 723-734.
- KLENK, H. D., ROTT, R., ORLICH, M., and BLODORN, J. (1975). Activation of influenza A viruses by trypsin treatment. *Virology* 68, 426-439.
- LAEMMLI, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)* 227, 680-685.
- LAZAROWITZ, S. R., COMPANS, R. W., and CHOPPIN, P. W. (1971). Influenza virus structural and non-structural proteins in infected cells and their plasma membranes. *Virology* 46, 830-846.
- LAZAROWITZ, S. G., and CHOPPIN, P. W. (1975). Enhancement of the infectivity of influenza A and B viruses by proteolytic cleavage of the hemagglutinin polypeptide. *Virology* 68, 440-454.
- LENARD, J., and COMPANS, R. W. (1974). The membrane structure of lipid-containing viruses. *Biochim. Biophys. Acta* 344, 51-94.
- MCINTOSH, K. (1974). Coronaviruses: A comprehen-

TABLE 2

PROPOSED DESIGNATION OF CORONAVIRUS STRUCTURAL PROTEINS

Apparent mw on SDS-PAGE ($\times 10^{-3}$)	Designation
23	E1
50	N
180/90	E2

- sive review. In "Current Topics in Microbiology and Immunology" (W. Arber *et al.*, eds), pp. 85-129. Springer-Verlag, New York.
- MENGELING, W. L. (1972). Precipitating antigens of hemagglutinating encephalomyelitis virus demonstrated by agar gel immunodiffusion. *Amer. J. Vet. Res.* 33, 1527-1535.
- SCHEID, A., and CHOPPIN, P. W. (1974). Identification of biological activities of paramyxovirus glycoproteins. Activation of cell fusion, hemolysis, and infectivity by proteolytic cleavage of an inactive precursor protein of Sendai virus. *Virology* 57, 475-490.
- SCOCCA, J., and LEE, Y. C. (1969). The composition and structure of the carbohydrate of pineapple stem bromelain. *J. Biol. Chem.* 244, 4852-4863.
- SKEHEL, J. J. (1972). Polypeptide synthesis in influenza virus-infected cells. *Virology* 49, 23-36.
- STRAUSS, J. H., JR., BURGE, B. W., and DARNELL, J. E. (1970). Carbohydrate content of the membrane protein of Sindbis virus. *J. Mol. Biol.* 43, 437-448.
- STURMAN, L. S. (1977). Characterization of a coronavirus. I. Structural proteins: Effects of preparative conditions on the migration of protein in polyacrylamide gels. *Virology* 77, 637-649.
- TANFORD, C. (1962). Contribution of hydrophobic interactions to the stability of the globular conformation of proteins. *J. Amer. Chem. Soc.* 84, 4240-4247.
- TEVETHIA, S. S., and CUNNINGHAM, C. H. (1968). Antigenic characterization of infectious bronchitis virus. *J. Immunol.* 100, 793-798.