Bovine coronavirus hemagglutinin protein

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Summary

Treatment of purified bovine coronavirus (Mebus strain) with pronase destroyed the integrity of virion surface glycoproteins gp140, gp120, gp100, reduced the amount of gp26 and destroyed the hemagglutinating activity of the virus. Bromelain, on the other hand, destroyed the integrity of gp120, gp100 and gp26 but failed to remove gp140 and failed to destroy viral hemagglutinating activity. These experiments suggest that gp140 is the virion hemagglutinin. Immunoblotting studies using monospecific antiserum demonstrate that gp140 is a disulfide-linked dimeric structure reducible to monomers of 65 kDa.

bovine coronavirus, hemagglutinin, bromelain resistance

Both hemagglutinating and nonhemagglutinating mammalian coronaviruses have been identified (McIntosh, 1974). When several mammalian coronaviruses were classified on the basis of antigenic relatedness it was found that the hemagglutinating coronaviruses could be classed into one of two major antigenic subgroups, although not all viruses within this subgroup had hemagglutinating properties (Pederson et al., 1978). Included in this subgroup were the hemagglutinating bovine enteric coronavirus, the porcine hemagglutinating encephalomyelitis virus, the hemagglutinating human respiratory coronavirus OC43, and the nonhemagglutinating mouse hepatitis virus 3. In this paper we report that the molecule responsible for hemagglutination by the bovine coronavirus is apparently one of four surface glycoproteins, gp140, which exists as a disulfide-linked dimer of two glycoproteins of 65 000 Da.

Plaque purified bovine coronavirus (Mebus strain) was grown either on the bovine embryo kidney cell line BEK-1 (Inaba et al., 1976) or on the human rectal tumor cell line HRT-18 (Tompkins et al., 1974) as described (King and Brian, 1982). Virus grown on either cell line was virtually identical as judged from electrophoretic
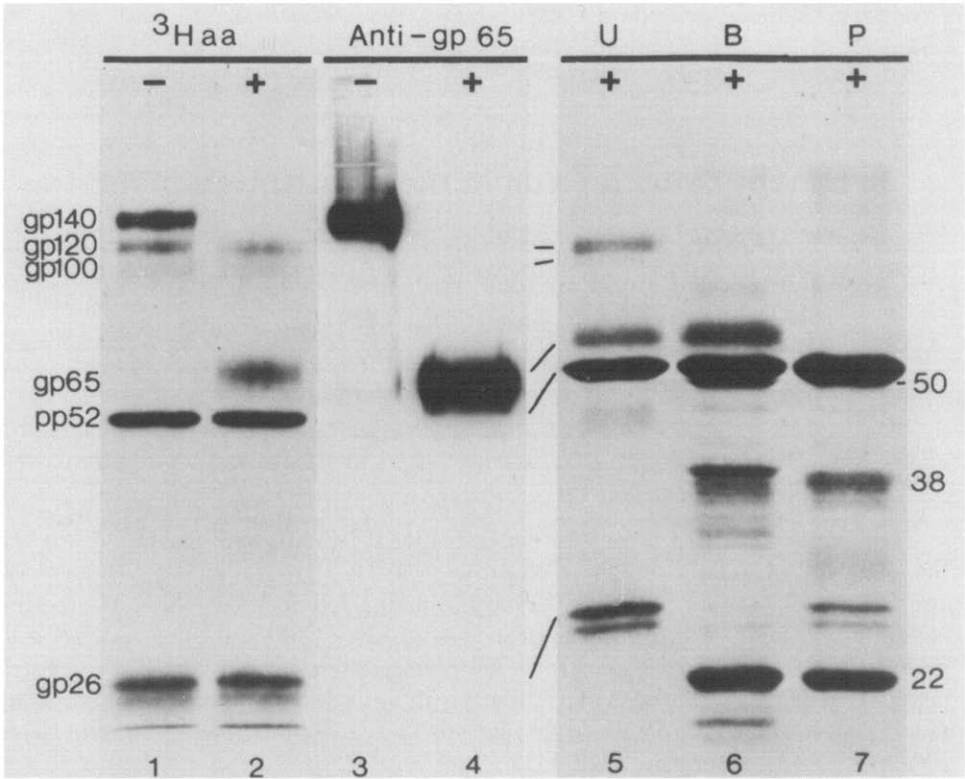
Fig. 1. Properties of the BCV gp140 protein. [³H]Amino acid-labeled BCV, purified as described (King and Brian, 1982), was electrophoresed on a 10% polyacrylamide gel in the absence of (lane 1) or after treatment with 2% 2-mercaptoethanol (lane 2), and fluorographed. Purified, unlabeled BCV was electrophoresed on a 9% polyacrylamide gel in the absence of (lane 3) or after treatment with 10% 2-mercaptoethanol (lane 4), and immunoblotted with rabbit antiserum prepared against gp65. [³H]Amino acid-labeled BCV recovered from the gradients depicted in Fig. 2 was electrophoresed on a 12% polyacrylamide gel after treatment with 2% 2-mercaptoethanol. Lane 5 is untreated control virus, lane 6 is bromelain-treated virus, and lane 7 is pronase-treated virus.

profiles of viral proteins. Virus, purified by isopycnic sedimentation as described (King and Brian, 1982), was solubilized in 0.0625 M Tris HCl, pH 6.8/2% SDS/5 M urea, electrophoresed by the method of Laemmli (1970), and immunoblotted by the method of Towbin et. al. (1979) as modified by Burnette (1981) except that proteins were transferred to nitrocellulose with a gradient of 100 V for 2 h and nitrocellulose wash buffer consisted of 150 mM NaCl/5 mM EDTA/50 mM Tris-HCl, pH 7.4/0.25% gelatin/0.5% Triton/0.1% SDS. Antiserum was prepared by immunizing separate rabbits with 144, 240, 552, and 264 μg each of SDS–polyacrylamide gel-purified gp120–gp100 (combined), gp65, pp52 and gp26 respectively. Proteins used for immunization were prepared from virus purified by isopycnic and rate-zonal sedimentation as previously described (King and Brian, 1982). Polyacrylamide plugs containing isolated proteins were minced, divided into three portions, and each
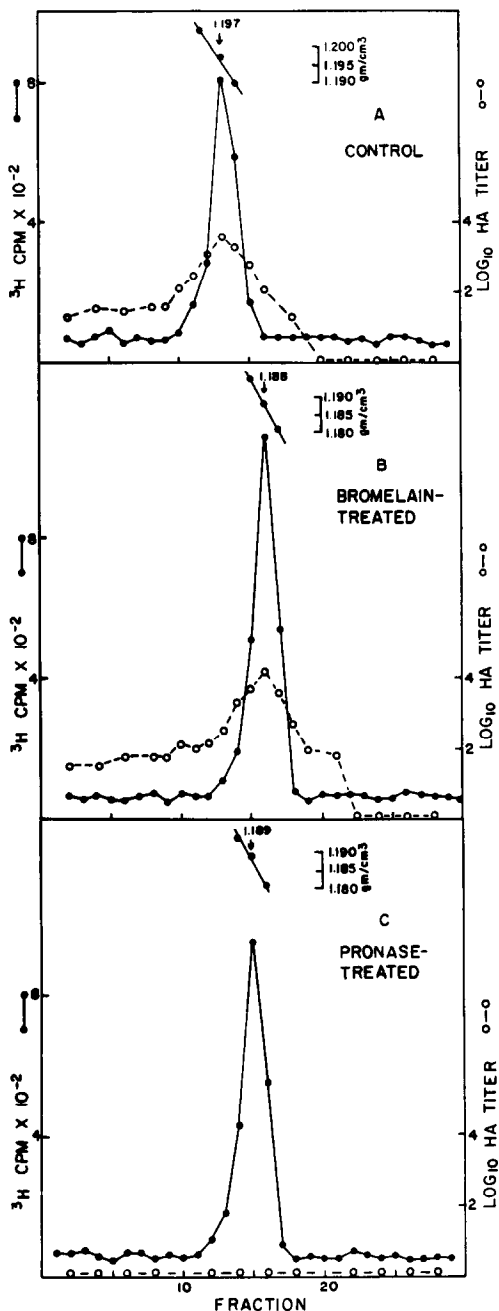
Fig. 2. Isopycnic sedimentation and hemagglutination by BCV following bromelain or pronase treatment. [^{3}H]Amino acid-labeled BCV was purified as described (King and Brian, 1982). One third was incubated in TMN buffer (50 mM Tris acid maleate, pH 6/100 mM NaCl) for 1 h at 37°C and resedimented isopycnically (panel A). One third was incubated in TMN containing 1.3 mg/ml bromelain (Sigma Chemical Co.) and 50 mM dithiothreitol for 1 h at 37°C, a modified procedure of Compans et al. (1970), and resedimented isopycnically (panel B). One third was treated with pronase for 1 h at 37°C as previously described (King and Brian, 1982) and resedimented isopycnically (panel C). The buoyant densities were determined from the refractive index of each fraction. 10 μl aliquots of each fraction were assayed for acid-precipitable radioactivity and hemagglutinating activity. Peak fractions of each gradient were separately pooled and analyzed by electron microscopy and gel electrophoresis.

portion was emulsified with 400 µg N-acetylmuramyl-L-alanyl-D-isoglutamine (Calbiochem) prior to subcutaneous injection. Single portion amounts were injected at 2 week intervals. Serum was diluted 1 : 10 for immunoblotting experiments. When each of the antisera was used in separate immunoblotting experiments, only antiserum prepared against gp65 reacted with gp140 under non-reducing conditions or with gp65 when 2-mercaptoethanol was included in the solubilization buffer (Fig. 1, and data not shown). Preimmune serum did not react with this protein nor did immune serum react with a protein from uninfected cells (data not shown). Gp65 and gp140 therefore behave as monomeric and dimeric forms of the same polypeptide supporting the tentative conclusion made in our earlier report (King and Brian, 1982).

While trying different proteases to remove the external proteins of BCV we observed that bromelain-treated virus still hemagglutinated mouse erythrocytes whereas pronase completely destroyed this activity (Fig. 2). The hemagglutinating titer of bromelain-treated virus was observed in nine experiments to range from 100 to 800% of that observed for an equal amount of untreated virus. Both enzymes decreased the buoyant density of the virus by approximately 0.01 gm/cm^3, however (Fig. 2). Since pronase was shown to remove or drastically reduce all surface glycoproteins of the virion (King and Brian, 1982), bromelain-treated and pronase-treated virus were analyzed by electrophoresis in parallel to determine which protein(s) might be responsible for hemagglutination. Virus radiolabeled with [^{3}H]amino acids and purified as described (King and Brian, 1982), was divided equally for the various treatments and resedimented isopycnically (Fig. 2). Electrophoretic analysis of particles from the peak regions of each gradient revealed that bromelain treatment altered the integrity of all glycoproteins excepting gp140, or gp65 when virus was electrophoresed with 2-mercaptoethanol, and left the internal protein pp52 intact (Fig. 1). The molar amounts of gp65 relative to internal pp52 remained the same after bromelain treatment (Table 1). In addition, several poly-

TABLE 1

RELATION BETWEEN gp65 AND PARTICLE HEMAGGLUTINATING ACTIVITY AFTER PROTEASE DIGESTION

	Relative molar amounts [a] or relative hemagglutinability [b]		
	Control	Bromelain-treated	Pronase-treated
gp65	0.21	0.22	0
gp120-100	0.07	0	0
pp52	1	1	1
gp26	1.36	0.11	0.25
Hemagglutinability	100%	300%	0%

[a] Molar amounts are relative to capsid protein as determined by densitometric scanning of fluorograms. Average of 3 experiments.

[b] Comparison of the peak hemagglutinating titer in the sucrose gradient of treated virus to the titer of control (untreated) virus. An equal amount of purified virus was used for each part of the experiment. Average of 3 experiments.
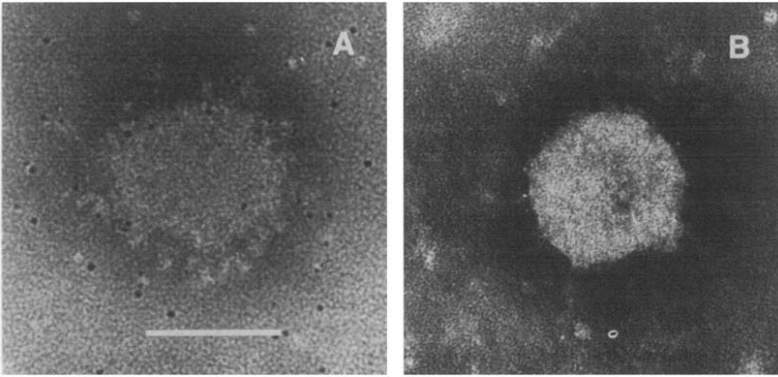
Fig. 3. Electron microscopy of bromelain-treated BCV. Virus recovered from the gradients depicted in Fig. 2 were analyzed by electron microscopy. 5 µl samples from the gradients were diluted with an equal volume of water, spotted onto Formvar-filmed, carbon-coated, glow-discharged copper grids, and stained with 2% phosphotungstic acid. Electron microscopy was done on a Philips 201. (A) Control virus. (B) Bromelain-treated virus. The bar represents 100 nm.

peptides migrating faster than pp52 appeared after bromelain treatment that resembled those observed after pronase treatment (King and Brian, 1982, Fig. 1), namely a minor 50 kDa species, a broad band of polypeptides in the region of 38 kDa, and an abundant 22 kDa species. We interpret these to be envelope-associated, digestion-resistant fragments of gp120, gp100 and gp26 that, on the basis of size alone, appear to represent nearly the same portion of the molecule regardless of the enzyme used. Further experimentation is needed to determine the exact origin of these species, however. These data, along with those demonstrating gp140, gp120, gp100 and gp26 to be surface glycoproteins (King and Brian, 1982), suggest that gp140 is the BVC hemagglutinin.

Electron microscopy revealed that purified untreated virions had irregularly spaced large bulbous peplomers as we previously reported (King and Brian, 1982) but that bromelain-treated virions appeared to have a fringe of very short projections (Fig. 3). The short projections may be the structural counterparts of gp140 but may also represent bromelain resistant fragments of other glycoprotein species. Projections of two different sizes have been reported for the bovine coronavirus (Bridger et al., 1978). Increased hemagglutinability following bromelain digestion could be explained by a removal of the large peplomers that sterically interfere with the interaction of erythrocytes and the hemagglutinin.

Consistent with gp140 being the hemagglutinin protein we find that the antigenically related hemagglutinating encephalomyelitis virus (unpublished data) and hemagglutinating human coronavirus OC43 (Hogue et al., 1984) also contain an antigenically related gp140 molecule that behaves as a disulfide-linked dimer of 65 kDa molecules. The non-hemagglutinating mouse hepatitis coronavirus A59, on the other hand, does not have this molecular counterpart as evidenced by direct electrophoretic analysis and immunoblotting studies using the rabbit anti-gp65 serum employed in the experiment described in Fig. 1 (Hogue et al., 1984). A report

by Callebaut and Pensaert (1980) also describes a 140 kDa disulfide-linked, dimeric glycoprotein for the hemagglutinating encephalomyelitis virus of swine, that, in a semipurified form, interferes with hemagglutination by this virus. These experiments also suggest the 140 kDa glycoprotein functions as the hemagglutinin molecule. Interestingly, in the studies of Callebaut and Pensaert, bromelain did destroy the hemagglutinating properties of the virus suggesting there may be some distinct structural differences between BCV and the porcine hemagglutinating encephalomyelitis virus.

Bromelain does not universally remove surface projections from enveloped viruses. Resistance of one or more peplomers was observed for the paramyxovirus SV5 (Chen et al., 1971), the porcine transmissible gastroenteritis virus (Garwes and Pocock, 1975), and the human coronavirus OC43 (Hierholzer et al., 1972). Perhaps bromelain sensitivity of the coronavirus hemagglutinin is not a common feature and can be used to further classify the hemagglutinating coronaviruses. The insensitivity of the BCV gp140 to digestion in our studies was not due to an idiosyncrasy of one specific lot of enzyme since the same results were obtained using two separate lots.

Experiments to demonstrate inhibition of hemagglutination with monospecific antiserum prepared against gp140, gp120–gp100 (together), pp52 or gp26 were not successful. This may be due to the fact that the epitopes for erythrocyte binding were destroyed by denaturants in the preparation of gp140 for antiserum production, since antiserum prepared against SDS-treated whole virus likewise does not inhibit hemagglutination.

Acknowledgements

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