Mapping of Neutralizing Epitopes to Fragments of the Bovine Coronavirus E2 Protein by Proteolysis of Antigen-Antibody Complexes

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SUMMARY

Neutralizing antigenic domains on bovine coronavirus gp100/E2 were mapped to fragments of this protein by proteolytic cleavage and fragment analysis. The procedure involved analysis of fragments generated after incubation of E2–monoclonal antibody complexes with various proteases. The smallest antibody-bound fragments obtained were a 50K fragment following Staphylococcus aureus V8 protease and submaxillary protease digestion, and a 37K fragment following trypsin digestion. Trypsin also produced a transient antibody-bound 50K fragment. A 40K fragment which was not bound by antibody was observed following digestions with all three proteases. The 50K fragments generated by V8, submaxillary protease and trypsin comigrated on gels and displayed the same altered mobility under non-reducing conditions, suggesting identity of these fragments and indicating the presence of disulphide linkages in these fragments. The 40K fragments generated by these three enzymes also comigrated and displayed the same altered mobility under non-reducing conditions. The 37K trypsin fragment contained both neutralizing domains, A and B.

INTRODUCTION

The E2 glycoprotein, the large peplomeric protein of coronaviruses, is involved in the adsorption of virus to cells and elicits the production of virus-neutralizing antibodies (Collins et al., 1982; Talbot et al., 1984; Wege et al., 1984). With the aid of monoclonal antibodies (MAbs), as many as eight neutralizing antigenic determinants have been identified on this protein for swine transmissible gastroenteritis virus (TGEV) (Delmas et al., 1986; Jiménez et al., 1986; Correa et al., 1988). For the bovine coronavirus (BCV) E2 protein, two neutralizing antigenic domains were defined using MAbs in competitive binding assays (Deregt & Babiuk, 1987).

The E2 glycoprotein (also designated as the spike or S protein) is observed as an uncleaved form of about 170K to 200K and/or cleaved forms of approximately 85K to 100K; the latter forms may either comigrate or migrate as separate entities on polyacrylamide gels (Sturman et al., 1985; Cavanagh, 1981; Stern & Sefton, 1982). Thus the 5' half of the E2 gene is known as the S1 region and the 3' half is known as the S2 region, yielding the S1 and S2 polypeptides, respectively, after proteolytic cleavage (Binns et al., 1985; Cavanagh et al., 1986). These two E2 species are held together in a non-covalent manner on the virion (Cavanagh, 1983).

To date, no antigenic determinants for any coronavirus E2 protein have been mapped to specific E2 gene sequences, although this gene from several coronaviruses has been sequenced and MAbs have been produced (Binns et al., 1985; Schmidt et al., 1987; Luytjes et al., 1987; Rasschaert & Laude, 1987; de Groot et al., 1987). However, Makino et al. (1987) have localized neutralizing determinants to the carboxy-terminal one-third of the E2 protein for mouse hepatitis virus (MHV) by analysis of RNA recombinants with crosses within the gene encoding
the pexipemic protein. Further, MAbs to TGEV have been shown to react with discrete proteolytic fragments of the TGEV E2 protein and thus epitopes may soon be mapped to the E2 gene of TGEV (Delmas & Laude, 1987). For avian infectious bronchitis virus, neutralizing MAbs produced against the E2 protein were found to bind to the S1 (non-anchoring) glycoprotein (Mockett et al., 1984; Niesters et al., 1987). The availability of BCV-neutralizing MAbs and the BCV E2 gene sequence (M. D. Parker et al., unpublished data) now make it feasible to produce a physical map of important epitopes on the BCV E2 protein.

The aim of this study was to identify these determinants and begin to relate that information to the structure of the BCV E2 protein. To attain this goal, a procedure that was successful in identifying antigenically active fragments of glycoprotein gD of herpes simplex virus was modified and used (Eisenberg et al., 1982). This procedure involved incubation of E2–MAb complexes with various proteases and subsequent identification of antibody-bound fragments. The smallest bound fragment of gp100/E2 that was identified was a 37K fragment produced by trypsin digestion. This fragment contained both group A and B neutralizing antigenic domains.

METHODS

Virus and cells. The Quebec isolate of BCV was grown in Madin–Darby bovine kidney (MDBK) cells as described previously (Deregt et al., 1987).

Isotopic labelling of intracellular proteins. BCV at an m.o.i. of 5 to 10 p.f.u./cell was allowed to adsorb to MDBK cells in 60 or 100 mm dishes for 2 h. At 23 to 25 h post-adsorption, BCV-infected and mock-infected cells were labelled for 30 min with 200 µCi/ml of [35S]methionine or 200 µCi/ml of a all-labelled amino acid mixture (a mixture of leucine, lysine, phenylalanine, proline and tyrosine), after incubation of cells in methionine- or amino acid-deficient medium for 2 h or 30 min, respectively, as described previously (Deregt et al., 1987). Both radioisotopes were from Amersham. Labelled cells were washed and harvested in ice-cold phosphate-buffered saline (for immunoprecipitation of gp170/pE2) or washed with Eagle’s MEM and then further incubated with MEM containing 100 times the normal concentration of unlabelled methionine or with medium containing five times the normal concentration of the unlabelled amino acids (leucine, lysine, phenylalanine and tyrosine) for 4 h before harvesting (for immunoprecipitation of gp100/E2).

Radioimmunoprecipitation and proteolysis of E2–antibody complexes. The procedure for immunoprecipitation of infected cell lysates with BCV E2-specific MAbs (ascites fluid) and rabbit anti-mouse IgG Immunobeads (Bio-Rad) was as described previously (Deregt et al., 1987), with the exception that immunoprecipitates were washed twice with RIPA buffer (0.05 M-Tris-HCl pH 7.0, 0.15 M-NaCl. 1% deoxycholate, 1% Triton X-100) containing 0.1% SDS without proteolytic inhibitors. The immunoprecipitates were then washed once with the appropriate enzyme buffer and incubated in 50 µl volumes with either Staphylococcus aureus V8 protease (ICN Biomedical) (usually 25 µg) in 50 mM-Tris–HCl pH 7.5; or trypsin (type XIII, TPCK-treated; Sigma) (usually 10 µg) in 100 mM-Tris–HCl pH 7.5. All incubations were carried out at 37 °C on a rocker platform (Nutator). Incubation periods generally ranged from 5 min to 6 h. Overnight digestions were occasionally analysed (18 to 24 h). The resulting proteolytic digests were then treated in one of two ways. For unfractionated digests the enzymic reaction was terminated by the addition of 25 µl of threefold concentrated sample buffer (Laemmli, 1970), with or without 2-mercaptoethanol, and boiling for 2 min; samples were then stored at −70 °C until analysis by PAGE. For fractionated proteolytic digests the incubation mixtures were pelleted by centrifugation at 13000 g for 5 min. The supernatant fraction containing unbound fragments of E2 was removed and treated as for unfractionated digests. The pelleted material containing rabbit anti-mouse IgG Immunobeads, MAb and bound fragments of E2 was washed twice with enzyme buffer. After suspension in sample buffer, samples were boiled for 2 min before storage at −70 °C. Before analysis by PAGE, unfractionated digests and pelleted fractions were centrifuged at 13000 g for 5 min to remove the Immunobeads.

Proteolysis of denatured gp100/E2. A modification of the procedure of Cleveland et al. (1977) was used. Briefly, 3H-amino acid-labelled, immunoprecipitated gp100/E2 was subjected to electrophoresis in 9% polyacrylamide gels. The preparative gel was then fixed in 7% acetic acid for 30 min before drying for 2 h at 60 °C. The gp100/E2 band was located by autoradiography and alignment of the gel with the autoradiograph. The gel slice, with backing paper, was inserted into the wells of the analytical gel and equilibrated with buffer containing 0.125 M-Tris–HCl pH 6.8, 0.1% SDS and 1 mM-EDTA before addition of enzyme and electrophoresis. PAGE. The procedure used was as previously described (Deregt et al., 1987). Proteolytic digests were analysed in both 10 and 15% polyacrylamide gels. After electrophoresis, gels were stained with Coomassie Brilliant Blue and fluorographed (Amplify; Amersham). After drying, gels were placed in contact with either Kodak XAR-5 film or 3M film (Picker International) and stored at −70 °C.
RESULTS

Proteolysis of antigen–antibody complexes

The properties of virus-neutralizing, E2-specific MAbs used in these experiments are listed in Table 1. These MAbs represent two antigenic groups based on competitive binding assays (CBAs) (Deregt & Babiuk, 1987). MAb JB9-3, which was not assigned to a particular antigenic group, showed intermediate levels of competition with conjugated MAb BB7-14 in a one-way CBA. All of these MAbs immunoprecipitate four polypeptide species from BCV-infected cell lysates: gpl00/E2, gpl90/E2, gpl70/pE2 (E2 precursor) and gp340, an apparent aggregate of gpl70 (Deregt et al., 1987; Deregt & Babiuk, 1987).

Initial experiments to map neutralizing epitopes by Western blotting of proteolytic fragments of the virion E2 protein were not successful, although these MAbs react to antigen denatured by SDS without 2-mercaptoethanol (Deregt & Babiuk, 1987). Since this method required relatively large quantities of antigen, and since proteolysis and transfer of fragments to nitrocellulose was not always efficient, an alternative method described by Eisenberg et al. (1982) for epitope mapping was modified and employed. This method involved proteolysis of antigen–antibody complexes. In theory, if an antibody can protect an epitope from proteolysis and if the cleaved fragment(s) bearing the epitope remains bound to intact antibody, the protein fragment(s) should be recoverable. Given these conditions, recovery of epitope-bearing fragments can be obtained by centrifugation, if these complexes are further bound to an insoluble support (rabbit anti-mouse IgG Immunobeads).

To examine the effect of proteolysis on the E2 protein (gp100) bound by MAb, BCV-infected cells were labelled with [35S]methionine or 3H-amino acids and then chased with excess unlabelled methionine or amino acids, respectively. After immunoprecipitation of cell lysates with E2-specific MAb and Immunobeads, the immune complexes were collected and resuspended in buffer containing either S. aureus V8 protease, submaxillary protease or trypsin. These enzymes cleave on the carboxyl side of glutamic acid (V8 protease), arginine (submaxillary protease), and both arginine and lysine (trypsin). After incubation with enzyme the resulting digests (unfractionated or separated into pellet and supernatant fractions) were analysed by PAGE. These proteases had little, if any, effect on bound IgG (as determined by Coomassie Brilliant Blue staining).

**Table 1. BCV E2 MAbs**

<table>
<thead>
<tr>
<th>Designation</th>
<th>Isotype</th>
<th>Group*</th>
<th>Neutralization†</th>
</tr>
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<tbody>
<tr>
<td>HB10-4</td>
<td>G1</td>
<td>A</td>
<td>+</td>
</tr>
<tr>
<td>JB5-6</td>
<td>G2a</td>
<td>A</td>
<td>+</td>
</tr>
<tr>
<td>HP9-8</td>
<td>G1</td>
<td>A</td>
<td>+</td>
</tr>
<tr>
<td>HE7-3</td>
<td>G1</td>
<td>A</td>
<td>+</td>
</tr>
<tr>
<td>BB7-14</td>
<td>G2b</td>
<td>B</td>
<td>+</td>
</tr>
<tr>
<td>JB9-3</td>
<td>G1</td>
<td>NA†</td>
<td>+</td>
</tr>
</tbody>
</table>

* Antigenic group determined by CBAs (Deregt & Babiuk, 1987).
† As determined by virus plaque reduction assays (Deregt & Babiuk, 1987).
‡ NA, Not assigned.
Fig. 1. Proteolysis of MAb-bound gp100/E2 by S. aureus V8 protease. Mock-infected (lane 1) and BCV-infected (lanes 2 to 12) cells were pulse-labelled for 30 min with 3H-amino acids and chased with excess unlabelled amino acids. Cell lysates were immunoprecipitated with MAb HB10-4. Immune complexes were collected after the addition of rabbit anti-mouse IgG Immunobeads (Bio-Rad). Immune complexes were then incubated with 0 µg (lanes 1, 2 and 12), 25 µg (lanes 3 to 9 and 11) or 100 µg (lane 10) of V8 protease for 5 min (lanes 1 to 3), 15 min (lane 4), 30 min (lane 5), 60 min (lane 6), 120 min (lane 7), 240 min (lane 8), 360 min (lanes 9, 10 and 12) and 18 h (lane 11). Samples were solubilized in Laemmli buffer containing 2-mercaptoethanol. Analysis was performed in a 10% polyacrylamide gel. Positions of $M_r \times 10^{-3}$ standards are indicated to the right of the gel.

result from the proteolysis of larger E2-related species (primarily gp170/pE2 and large aggregates). These bands, also seen in digestions with submaxillary protease and trypsin, were ignored for mapping purposes and are not discussed.

To determine the extent to which denatured gp100/E2 could be cleaved by V8 protease, a modification of the method of Cleveland et al. (1977) was employed. Proteolysis by this method produced a larger number of discrete fragments, including fragments of approximately 67K, 50K and 40K (not shown). Thus, it was apparent that the denatured gp100/E2 protein contained more sites cleavable by V8 protease than did the 'native' gp100/E2 protein bound by MAb HC10-4.

To determine which V8 fragments of gp100/E2, if any, remained bound to MAb HB10-4 and which fragments were unbound the digest was fractionated by centrifugation. These results are shown in Fig. 2. Both the 67K and 50K fragments were found in the pelleted fraction and thus were bound by MAb HB10-4, while the 40K fragment appeared in the supernatant and thus was not bound to MAb HB10-4.

When [35S]methionine was used to label gp100/E2, fragments in the size range of the 14.5K and 15.5K fragments were usually not observed in digests (Fig. 2). When they did occur, the 40K...
Mapping of neutralizing epitopes on BCV E2

Fig. 2. Proteolysis of MAb-bound gp100/E2 by S. aureus V8 protease: analysis of fractionated digests. BCV-infected cells were pulse-labelled with [35S]methionine and chased with excess unlabelled methionine. Cell lysates were immunoprecipitated as described in Fig. 1. Immune complexes were then incubated with 0 μg (lane 1), 25 μg (lanes 2 to 15, 18 and 19) or 100 μg (lanes 16 and 17) of V8 protease for various time periods. The immune complexes were then separated into pelleted (lane 1 and even-numbered lanes) and supernatant fractions (odd-numbered lanes, except lane 1). Incubation times were 5 min (lanes 1 to 3), 15 min (lanes 4 and 5), 30 min (lanes 6 and 7), 60 min (lanes 8 and 9), 120 min (lanes 10 and 11), 240 min (lanes 12 and 13), 360 min (lanes 14 to 17) and 18 h (lanes 18 and 19). Samples were solubilized in Laemmli buffer containing 2-mercaptoethanol. Analysis was performed in a 10% polyacrylamide gel.

Fragment was also observed to have undergone further proteolysis. Thus, the derivation of these minor fragments could be confused, but apparently there were both methionine-containing and methionine-lacking fragments in this size range. This requires further investigation. V8 digests utilizing other group A MAbs, BB7-14 (antigenic group B) and JB9-3 for immunoprecipitation yielded fragments of the same size in both pelleted and supernatant fractions as was observed for MAb HB10-4 (not shown).

Proteolysis of E2–MAb complexes with submaxillary protease or trypsin

The effects of proteolysis by submaxillary protease and trypsin on gp100/E2 bound by MAb HB10-4 are shown in Fig. 3 and 4 respectively. Digestion with submaxillary protease produced fragments of 86K, 50K and 40K. After fractionation, the 86K and 50K fragments were found in the pelleted fraction, while the 40K fragment was in the supernatant (Fig. 3). Digestion with trypsin produced fragments of 97K, 86K, 50K, 40K, 37K and 15K. After fractionation the trypsin-generated 97K, 86K, 50K and 37K fragments were found in the pelleted fraction and thus were bound to MAb HB10-4, while the 40K and 15K fragments appeared in the supernatant (Fig. 4).
Submaxillary protease and trypsin digests of gp100/E2 utilizing other group A MAbs or MAb BB7-14 (group B) again yielded fragments of the same size in pelleted and supernatant fractions as those observed for MAb HC10-4. This indicated that epitopes defined by group A and B MAbs were located relatively close to each other (i.e. both antigenic regions were located on the 37K tryptic fragment). The results of these experiments and the apparent cleavage pathways for each of the three proteolytic enzymes are summarized in Fig. 5.

Comigration of proteolytic fragments and the effect of reducing and non-reducing conditions on fragment mobility

All three proteolytic enzymes produced fragments of approx. 50K and 40K which could be separated into the pelleted fraction and supernatant respectively. To determine whether these fragments would actually comigrate and the effect of reducing and non-reducing conditions on fragment mobility, digests were prepared in sample buffer, with or without 2-mercaptoethanol, and subjected to electrophoresis on the same polyacrylamide gel. The 50K and the 40K fragments generated by the three enzymes comigrated under reducing conditions (Fig. 6a, lanes 1 to 6). Under non-reducing conditions, the 50K and 40K fragments, generated by all three
Mapping of neutralizing epitopes on BCV E2

Fig. 4. Proteolysis of MAb-bound gp100/E2 by trypsin: analysis of fractionated digests. The procedure for labelling of BCV-infected cells and immunoprecipitation of cell lysates is described in Fig. 2. Immune complexes were incubated with 0 μg (lane 1), 10 μg (lanes 2 to 15, 18 and 19) or 25 μg (lanes 16 and 17) of trypsin for various time periods. The immune complexes were separated into pelleted (lane 1 and even-numbered lanes) and supernatant fractions (odd-numbered lanes, except lane 1). Incubation times were 5 min (lanes 1 to 3), 15 min (lanes 4 and 5), 30 min (lanes 6 and 7), 60 min (lanes 8 and 9), 120 min (lanes 10 and 11), 240 min (lanes 12 and 13), 360 min (lanes 14 to 17) and 19 h (lanes 18 and 19). Samples were solubilized in Laemmli buffer containing 2-mercaptoethanol. Analysis was performed in a 10% gel.

Fig. 5. Summary and apparent cleavage pathway in the generation of the fragments observed from S. aureus V8 protease (a), submaxillary protease (b) and trypsin (c) digestion of MAb-bound gp100/E2. Numbers refer to the Mr of the observed fragments. Fragments expected but not observed are denoted by question marks. The 15.5K fragment (Fig. 1) was tentatively identified as the fragment resulting from V8 digestion in the generation of the 50K species.
enzymes, migrated with a greater mobility than under reducing conditions indicating the presence of disulphide linkages in these fragments (Fig. 6b, lanes 1 to 6). The 50K and 40K fragments generated by the three enzymes under these conditions had apparent $M_r$ values of $43K$ and $36K$ respectively. Other fragments, including the 37K tryptic fragment, were also observed to have altered mobilities when 2-mercaptoethanol was omitted from the sample buffer (Fig. 6).

Comparison of proteolytic digests of MAb-bound gp170/pE2 and gp100/E2

To determine the contribution of fragments from gp170/pE2 in gp100/E2 digests and the resulting fragment sizes derived by proteolysis of MAb-bound gp170/pE2, BCV-infected cells were pulse-labelled with $^{[35}S]$methionine and lysates were immunoprecipitated with MAb HB10-4 before a 6 h incubation with protease. The resulting digests of gp170/pE2 were then fractionated and compared with fractionated digests of MAb-bound gp100/E2 (Fig. 7).
Mapping of neutralizing epitopes on BCV E2

Fig. 7. Comparison of fragments derived from MAb-bound gp170/pE2 and gp100/E2 by digestion with V8 protease, submaxillary protease and trypsin: analysis of fractionated digests. BCV-infected cells were pulse-labelled with [35S]methionine (for gp170, lanes 1 to 6) or pulse-labelled with [35S]methionine and chased with excess unlabelled methionine (for gp100, lanes 7 to 12). The same procedure for immunoprecipitation of cell lysates described in Fig. 2 was then used. Immune complexes were digested for 6 h with 100 μg of V8 protease (lanes 1, 2, 7 and 8), 25 μg of submaxillary protease (lanes 3, 4, 9 and 10) or 25 μg of trypsin (lanes 5, 6, 11 and 12). Samples were solubilized in Laemmli buffer containing 2-mercaptoethanol. Pelleted fractions (odd-numbered lanes); supernatant fractions (even-numbered lanes). Analysis was done in a 10% polyacrylamide gel. Arrowheads alongside lane 1 indicate unique fragments of gp170 or fragments that may be attributed to gp170 in gp100 digests. Positions of Mr standards are depicted by arrowheads alongside lane 7, and are from top to bottom 92-5K, 66K, 45K, 31K and 21.5K. Lanes 1 and 2 were exposed longer than other lanes to achieve better resolution of bands.

V8 protease generated from antibody-bound gp170/pE2 many fragments that fractionated in the pellet, including a number of fragments that comigrated with fragments derived from digestions of gp100/E2 and a number of smaller fragments (Fig. 7, lane 1). It is suspected that the majority of these smaller fragments are actually disulphide-linked to larger epitope-bearing fragments; however, this requires further investigation. In addition, a number of fragments, including a 40K fragment, were observed in the supernatant (Fig. 7, lane 2). Some of the smaller fragments occurring in the supernatant fraction were apparently derived from further V8 digestion of the 40K fragment.

Submaxillary protease generated fragments from antibody-bound gp170/pE2 of 50K and 40K, which were found to occur in the pelleted fraction and supernatant respectively (Fig. 7, lanes 3 and 4). Treatment of antibody-bound gp170/pE2 with trypsin generated a 37K fragment found in the pelleted fraction and a 40K fragment found in the supernatant (Fig. 7, lanes 5 and 6). A trypsin-generated fragment of 52K also appeared in the supernatant fraction after shorter incubation times (not shown). This latter fragment was not observed in the supernatant fraction of gp100/E2 digests.
The results indicate that the smallest gp100/E2 fragment obtained, bearing neutralizing antigenic domains (of both group A and B MAbs), was a 37K fragment generated by trypsin digestion. Thus, the method of mapping employed was successful in that a relatively small, defined, epitope-bearing fragment could be obtained. The 50K tryptic fragment (from which the 37K fragment was generated) was very probably derived by cleavage at arginine residues (rather than lysine residues), as the cleavage pattern for submaxillary protease which is arginine-specific was very similar (both generated an 86K fragment before generating a 50K fragment). The final cleavage by trypsin to generate the 37K fragment from the 50K fragment probably occurred at a lysine residue (rather than at an arginine residue) for the opposite reason, i.e. failure of submaxillary protease to generate this fragment.

Digestion of gp100/E2 with all three proteases generated an antibody-bound 50K fragment and a non-bound 40K fragment. It is likely that the 50K fragments (and likewise the 40K fragments), generated by digestion with the three enzymes used, contain the same sequences, since these fragments have similar Mr values, fractionate to the same pool of fragments and display the same altered mobility in PAGE under non-reducing conditions. Further, it is apparent from the latter observation that these fragments (and the 37K tryptic fragment) contain disulphide bonds. Previously, we had determined that neutralizing (group A and B) MAbs were reacting with epitopes that were dependent upon disulphide bonds for their antigenic integrity (Deregt & Babiuk, 1987).

In proteolysis of MAb-bound gp100/E2 some protein remained resistant to digestion. The reason for this is not certain. Nevertheless, when SDS (0.05% final concentration) was added to the incubation buffer, gp100/E2 was completely cleaved by V8 protease to the 67K fragment within 5 min (results not shown). Addition of SDS also had the effect of promoting some proteolysis of IgG, although this occurred slowly. Finally, the 50K fragment was not observed in V8 digestions when SDS was added to the incubation buffer. Instead, a 42K fragment was observed in the pelleted fraction but only when this fraction had been reduced by the addition of 2-mercaptoethanol. Cleavage of the 67K fragment to the 42K fragment may have occurred following exposure of an additional V8 cleavage site by partial SDS denaturation. However, the 50K fragment was also not observed in a V8 digestion of MAb-bound gp100 when the pH of the incubation buffer (without SDS) was slightly lowered (by 0.35 units) in an initial experiment. Taken together these results cast some doubt on whether the 50K fragment found in digestions with V8 protease is indeed due to cleavage by this enzyme; rather, the possibility that a contaminating protease is involved in this cleavage can not be readily discounted.

There are some obvious advantages to epitope mapping by proteolysis of antigen–antibody complexes when compared to Western immunoblotting of proteolytic fragments. First, the amount of antigen required by the former method is small compared to that usually required for the latter. Second, in Western immunoblotting the antigen is usually denatured before SDS-PAGE, which can result in destruction of the epitope. In proteolysis of antigen–antibody complexes, the ‘native’ protein is used. Third, proteolytic cleavage of the antigen before Western immunoblotting may destroy the epitope and lead to negative results, if cleavage occurs at sequences critical to the integrity of the epitope. In proteolysis of antigen–antibody complexes, the antibody may protect the epitope from proteolysis. However, it should be noted that in proteolysis of antibody-bound proteins, fragments that fractionate with pelleted material but which are observed only under reducing conditions may, in fact, not be epitope-bearing but rather separate into the pelleted fraction because they are disulphide-linked to other epitope-bearing fragments. Thus, only fragments that fractionate in the pellet and are observed under both reducing and non-reducing conditions can definitely be assigned to the pool of epitope-bearing fragments. In proteolysis of E2 this was kept in mind and analysis was carried out under both conditions.

Proteolysis of MAb-bound gp170/pE2 with V8 protease generated some fragments that were smaller than those observed for gp100/E2 in pelleted fractions under reducing conditions. It remains to be seen whether these small fragments are also observed under non-reducing conditions and bear epitopes. It was expected that more fragments would be present in the
supernatant fraction after proteolysis of MAb-bound gp170/pE2. Only trypsin digestion of MAb-bound gp170 generated a transient 52K fragment in the supernatant, which was not observed in gp100/E2 digests in initial experiments using shorter incubation times. It is likely that fragments not bound by MAb in digestion experiments are more susceptible to further proteolysis than fragments bound by MAb. The lack of additional discrete fragments in supernatant fractions of gp170/pE2 digests may have been due to more extensive proteolysis resulting in heterogeneity of fragment size.

Based on the proteolytic cleavage patterns observed with the three enzymes used and on some preliminary data we have tentatively mapped the 37K tryptic fragment to a portion of the S1 region of the BCV E2 gene (results not shown). The location (to be confirmed by more definitive studies we have tentatively mapped the 37K tryptic fragment to a portion of the S1 protein. It is likely that the region contains a large unique sequence when compared to the E2 gene of MHV-JHM, similar to, but larger than that recently shown for MHV-A59 (Schmidt et al., 1987; Luytjes et al., 1987; M. D. Parker et al., unpublished data). Studies are in progress to determine whether this region is the actual location of neutralizing epitopes defined by E2-specific MAb.

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Mapping of neutralizing epitopes on BCV E2


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