Surface Structure and RNA-Protein Interactions of Foot-and-Mouth Disease Virus

By D. J. Morrell,† E. J. C. Mellor,‡ D. J. Rowlands§ and F. Brown*§

The Animal Virus Research Institute, Pirbright, Woking, Surrey GU24 0NF, U.K.

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SUMMARY

The surface structure of foot-and-mouth disease virus (FMDV) and the interaction of the individual capsid proteins with the virus RNA have been examined using modification reagents. By measuring the extent of modification of the lysine residues of intact and disrupted virus particles and the 12S protein subunit with Bolton & Hunter reagent it was found that 54% of the residues of VP1, 15% of the residues of VP2 and 37% of the residues of VP3, equivalent to five, two and four lysine residues respectively, are on the surface of the intact virus particle. Polypeptide VP4 was not modified in intact virus particles, indicating that it has no lysine residues on the surface of the virus. Modification with sodium metabisulphite, which causes a specific transamination reaction between cytidylic acid residues in ssRNA and closely associated basic amino acids, cross-linked all four structural proteins to the virus RNA. Both fragments of VP1, produced by treatment of the virus particle with trypsin, are also cross-linked to the RNA. These observations have been combined with the evidence that the immunogenic activity of VP1 may be contained in two discontinuous sites, at amino acids 141 to 160 and 200 to 213, in proposing a model for the arrangement of this polypeptide in the virus particle.

INTRODUCTION

Foot-and-mouth disease virus (FMDV) forms the genus aphthovirus of the family Picornaviridae (Cooper et al., 1978). The virus particles are composed of 60 copies of four structural proteins, VP1 to 4 (mol. wt. values: VP1 to 3 approx. 24 × 10^3, VP4 approx. 8.5 × 10^3; Boothroyd et al., 1982), which form a protein capsid surrounding a single positive-strand RNA molecule, mol. wt. 2.8 × 10^6. The aphthoviruses break down under mild acid conditions (pH 6.5) to give VP4, the virion RNA and a 12S subunit containing five copies of the structural proteins VP1 to 3 (Vasquez et al., 1979; Morrell, 1983).

The use of protein modification reagents to examine the surface of picornavirus particles can give important information concerning the virus structure. Such studies have been performed on FMDV using lactoperoxidase-catalysed iodination (Laporte & Lenoir, 1973; Talbot et al., 1973). However, comparison of the results of chemical modification of proteins with their three-dimensional structures obtained by X-ray diffraction studies has shown that lysine modification gives a better indication of surface exposure than tyrosine modification (Glazer, 1982). To obtain a greater understanding of the structure of the FMDV particle the surface exposure of the structural polypeptides of the virus and 12S particles has been examined with Bolton & Hunter reagent [N-succinimidyl-3-(4-hydroxy-5-iodophenyl)propionate; Bolton & Hunter, 1973], a small lysine-modifying reagent.

† Present address: Department of Growth and Development, Institute of Child Health, 30, Guilford Street, London WC1N 1EH, U.K.
‡ Present address: Department of Biochemistry, South Parks Road, Oxford OX1 3QU, U.K.
§ Present address: Wellcome Biotechnology Limited, Ash Road, Pirbright, Woking, Surrey GU24 0NQ, U.K.
The presence of intact RNA within the virus particle stabilizes the capsid against degradation (Bachrach, 1968; Denoya et al., 1978a) suggesting that the RNA is involved in interactions with the protein components of the virus capsid. These interactions have been studied by covalently cross-linking basic amino acids of the capsid proteins to cytidylic acid residues of the virus RNA in situ using sodium metabisulphite (Shapiro & Gazit, 1977). In addition, production of RNA-free 'artificial empty' or 12S particles from mature FMDV particles results in a change of antigenicity (Rowlands et al., 1975; Cartwright et al., 1982). Thus, maintenance of the correct conformation of the antigenic determinants appears to be RNA-dependent. The antigenic determinants of FMDV have been shown to be located within VP1 (Laporte et al., 1973; Strohmaier et al., 1982; Bittle et al., 1982). To locate more precisely the relationships between the antigenic and RNA-binding sites of VP1, advantage was taken of the fact that trypsin cleaves the protein in situ at defined positions to give two major fragments L and S (Burroughs et al., 1971), whose participation in RNA-protein cross-links following bisulphite treatment was examined.

The interactions of the structural proteins of FMDV with the internal and external environments have been studied. The implications of this work for virus structure, stability and antigenicity are discussed.

METHODS

Virus growth and purification. Monolayers of BHK-21 cells in Roux flasks (about 10⁸ cells/flask) were infected with FMDV of serotype A, subtype 10, strain Argentina 1961 (A61) and the virus particles were radioactively labelled by the addition to the culture medium of [³²S]methionine (150 μCi/flask), [³H]leucine (300 μCi/flask) or [³H]uridine (500 μCi/flask). For labelling with amino acids, medium lacking the appropriate amino acid was used. All radiochemicals were obtained from Amersham. Virus was purified by differential centrifugation, treatment with 10⁻¹ (w/v) SDS and sucrose density centrifugation (Brown & Cartwright, 1963; Harris & Brown, 1977).

Preparation of 12S particles. An equal volume of 0.2 M-citric acid, 0.1 M-NaCl was added to 0.5 ml of a sucrose density gradient fraction containing purified [³H]leucine-labelled virus particles (approx. 150 μg/ml). Pancreatic RNase A (Sigma), 2 mg/ml in 0.3 M-sodium acetate buffer pH 5.0, was added at a final concentration of 100 μg/ml and the mixture was incubated at 37 °C for 30 min. After filtration through a 10 ml Sephadex G-50 column equilibrated with TN buffer (0.1 M-NaCl, 0.1 M-Tris-HCl pH 7.4), the crude 12S particle preparation was centrifuged through a 20 ml 15 to 25% (w/v) sucrose gradient in TN buffer for 16 h at 30000 r.p.m. (MSE 3 × 23 rotor). Fractions (1 ml) were collected and the 12S particles were detected by liquid scintillation counting of samples of the fractions.

Modification with ¹²⁵I-labelled Bolton & Hunter reagent. Purified virus or 12S preparations were filtered through Sephadex G-25 columns equilibrated with 0.1 M-NaCl, 0.2 M-sodium borate buffer pH 8.2, concentrated by pressure dialysis against the same buffer and adjusted to 1 mg protein/ml. Disrupted virus was prepared by the addition of SDS and 2-mercaptoethanol (final concentrations 1% w/v and 2% v/v respectively) to a concentrated, purified virus preparation and boiling for 5 min.

¹²⁵I-labelled Bolton & Hunter reagent (1000 Ci/mmol; Amersham) was transferred to siliconized Eppendorf tubes (300 μCi/tube) and the solvent was removed by evaporation in a stream of dry nitrogen. Samples (25 μg) to be iodinated were added, with mixing, and the tubes were incubated at 20 °C for 30 min. The reaction was stopped by the addition of 100 μl 0.1 M-glycine in TN buffer. Following a further 30 min incubation the samples were passed through 10 ml Sephadex G-25 columns equilibrated with TN buffer to remove unreacted ¹²⁵I-labelled Bolton & Hunter reagent. Modified disrupted virus particles were precipitated with acetone. Modified virus or 12S particles were repurified by sucrose density centrifugation and precipitated with acetone.

Treatment with sodium metabisulphite. One M-sodium metabisulphite (1.4 ml), buffered with sodium sulphate and sodium hydroxide to pH 7.2 and containing 0.005 M-quinol as a free radical scavenger, was added to a siliconized Eppendorf tube containing 30 μl of a purified virus particles preparation labelled with both [³⁵S]methionine and [³H]uridine. After incubation at 37 °C for 4 h the reaction mixture was passed through a 10 ml Sephadex G-25 column equilibrated with TN buffer. Intact virus particles were repurified by sucrose density gradient centrifugation.

Trypsin digestion of bisulphite-treated virus particles. Repurified, bisulphite-treated particles were passed through a Sephadex G-25 column equilibrated with 0.04 M-sodium phosphate buffer pH 7.6, and incubated at 37 °C for 30 min in the presence of 100 μg/ml TPCK-trypsin (Worthington). The digestion was stopped by the RNA extraction procedure.

Extraction and purification of protein-RNA complexes. Bisulphite-modified virus particles were incubated at 56 °C for 5 min in 0.1 m-sodium acetate buffer pH 5.0, containing 0.1% (v/v) 2-mercaptoethanol and 0.05% (w/v)
RNA–protein interactions in FMDV

Fig. 1. SDS–PAGE of proteins from (a) disrupted virus particles, (b) 12S particles and (c) intact virus particles treated with $^{125}$I-labelled Bolton & Hunter reagent. Electrophoresis was from left to right.

SDS and centrifuged through 36 ml 5 to 25% sucrose density gradients in 0.14 M-NaCl, 0.005 M-EDTA, 0.5% (w/v) SDS, 0.05 M-Tris–HCl pH 7.6, for 16 h at 18000 r.p.m. (Beckman SW28 rotor) at 22 °C. Yeast tRNA was added to RNA sedimenting at about 37S and the mixture was precipitated with 2.5 vol. of ethanol at −20 °C overnight.

Preparation of samples for gel electrophoresis. Acetone-precipitated samples were resuspended in gel sample buffer (Laemmli, 1970) and boiled for 5 min. Ethanol-precipitated samples containing RNA were resuspended in 0.005 M-EDTA, 0.05 M-Tris-HCl pH 7.6. Half of each sample was treated with 500 units/ml RNase T1 (Sankyo) and 700 units/ml RNase A (Worthington) at 37 °C for 30 min. An equal volume of 2× concentration gel sample buffer was added to the samples which were then incubated at 56 °C for 15 min.

SDS–polyacrylamide gel electrophoresis (SDS–PAGE). Samples were loaded onto 10% discontinuous SDS-polyacrylamide gels (Laemmli, 1970) containing 8 M-urea (Vande Woude et al., 1972) in 0.5 × 12 cm tubes. Electrophoresis was at 20 mA until the bromophenol blue dye had migrated approx. 10 cm. The gels were fixed in 10% acetic acid, frozen and cut into 1 mm sections using a gel slicer (Mickle Instruments Ltd). Slices from gels containing $^{125}$I-labelled proteins were counted in a gamma counter. Slices from gels containing $^{35}$S-labelled proteins were digested in 0.2 ml NCS Tissue Solubilizer (New England Nuclear) at 37 °C for 4 h. Scintillation fluid was added and the slices were counted in a liquid scintillation counter.

RESULTS

Modification with $^{125}$I-labelled Bolton & Hunter reagent

Modified virus and 12S particles were repurified by sucrose density centrifugation. No evidence of particle breakdown was observed (results not shown). Fig. 1(a) shows the incorporation of $^{125}$I-labelled Bolton & Hunter reagent into the proteins of totally disrupted virus particles. The major structural proteins, VP1 to 3, were labelled in proportion to their lysine content (Table 1). It was not possible to quantify the incorporation of label into VP4 as this protein ran close to the solvent front. The label detected at higher mol. wt. is due to incorporation into minor structural components of the virus particle (Sangar et al., 1976).

Fig. 1(b) shows the incorporation of $^{125}$I-labelled Bolton & Hunter reagent into the proteins of 12S particles, which do not contain VP4. The extent of modification is essentially the same as in totally disrupted virus particles, demonstrating that all the lysine residues of 12S particles are externally located.

Fig. 1(c) shows the incorporation of $^{125}$I-labelled Bolton & Hunter reagent into the proteins of intact virus particles. The extent of modification is quantitatively and qualitatively different from that of 12S and totally disrupted virus particles. There is generally less incorporation and, in particular, the incorporation into VP2 is much decreased. After taking into consideration the level of radioactivity associated with the solvent front, derived from the labelling profile of 12S particles (Fig. 1b) which do not contain VP4, there is no detectable incorporation of Bolton & Hunter reagent into VP4 in intact virus particles.
Table 1. Incorporation of 125I-labelled Bolton & Hunter reagent into the structural proteins of FMDV

<table>
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<tr>
<th>Preparation</th>
<th>Protein</th>
<th>Label incorporated into disrupted virus particles (%)</th>
<th>No. of lysine residues in protein</th>
<th>Lysine residues labelled</th>
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<tr>
<td>Disrupted virus particles</td>
<td>VP1</td>
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<tr>
<td></td>
<td>VP2</td>
<td>100</td>
<td>13</td>
<td>13.0</td>
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<tr>
<td></td>
<td>VP3</td>
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<tr>
<td></td>
<td>VP4</td>
<td>ND</td>
<td>1</td>
<td>ND</td>
</tr>
<tr>
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<td>VP1</td>
<td>95</td>
<td>9</td>
<td>8.6</td>
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<td></td>
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<td>1</td>
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</tr>
</tbody>
</table>

* Derived from Fig. 1.
† From Boothroyd et al. (1982).
‡ 12S particles do not contain VP4.
§ ND, Not determined.
|| None detected.

Table 2. Cross-linking of the structural proteins of FMDV to the virus RNA

| Protein | Radioactivity in virus cross-linked to RNA (%, total)* | Methionine residues cross-linked to RNA per virus particle‡ | Number of methionine residues in protein§ | Protein molecules cross-linked to RNA per virus particle | Lysine residues available for cross-linking per virus particle$ | Efficiency of cross-linking $×10^3$||
|---------|------------------------------------------------------|--------------------------------------------------------|------------------------------------------|--------------------------------------------------------|------------------------------------------------|------------------||
| VP1     | 0.45¶                                                | 3.2                                                   | 3                                        | 1.07                                                  | 240                                           | 4.5              |
| VP1(L)  | 0.32**                                               | 2.3                                                   | 2                                        | 1.15                                                  | —                                              | —                |
| VP1(S)  | 0.18**                                               | 1.3                                                   | 1                                        | 1.30                                                  | —                                              | —                |
| VP2     | 1.67¶                                                | 12.0                                                  | 4                                        | 3.00                                                  | 660                                           | 4.5              |
|         | 1.68**                                               | 12.1                                                  | 3                                        | 3.03                                                  | —                                              | —                |
| VP3     | 0.38¶                                                | 2.7                                                   | 3                                        | 0.90                                                  | 360                                           | 2.5              |
|         | 0.40**                                               | 2.9                                                   | 2                                        | 0.97                                                  | —                                              | —                |
| VP4     | 0.63¶                                                | 4.5                                                   | 2                                        | 2.25                                                  | —                                              | —                |
|         | 0.58**                                               | 4.2                                                   | 2                                        | 2.1                                                   | —                                              | —                |

* Derived from Fig. 2; the radioactivity cross-linked was 3.1% and 3.2% of the total radioactivity in the virus preparation.
† Each methionine residue is equivalent to 0.14% of the total radioactivity.
‡ From Boothroyd et al. (1982).
§ [Lysine residues in protein (Boothroyd et al., 1982) minus lysine residues of protein on the surface of the virus (from Table 1)] × 60.
|| Protein molecules cross-linked to the RNA per lysine residue.
¶ Experiment 1.
** Experiment 2.

Assuming the same incorporation per lysine residue per protein molecule in virus particles, 12S particles and totally disrupted virus particles, the results indicate that 54% of the lysine residues of VP1, 15% of the lysine residues of VP2 and 37% of the lysine residues of VP3 are available for modification on the surface of the virus particle. This is equivalent to five lysine residues of VP1, two of VP2 and four of VP3 (Table 1). The results suggest that VP4 is not available for reaction on the surface of the virus particle.

The structural proteins VP1 to 3 have lysine residues exposed on the surface of 12S particles that are not exposed on the surface of the intact virus particle. The increased modification of 12S particles gives a measure of the number of lysine residues available for cross-link formation with the virion RNA upon bisulphite treatment (Table 2).
Protein–RNA cross-linking with sodium metabisulphite

Incubation at 37 °C for 4 h caused some degradation of virus particles in both untreated and bisulphite-treated preparations. A substantial proportion of the RNA from intact, repurified virus particles was also degraded (results not shown). Analysis by SDS–PAGE of the protein associated with the virus RNA was confined to full length RNA from intact virus particles. This constituted approx. 15% of the initial RNA.

To differentiate between proteins covalently cross-linked to the RNA and those non-covalently associated with the RNA, samples of RNA–protein complexes were analysed by SDS–PAGE either (i) directly, to show non-covalent association, since proteins linked to the RNA do not enter the gel, or (ii) after ribonuclease treatment, to show the total of covalently linked and non-covalently associated protein. The difference between the two profiles shows the extent of cross-linkage.

PAGE analysis of the RNA from untreated virus showed some non-covalently associated protein, but the level was very low (less than 300 d.p.m. per slice). This did not increase when the RNA was treated with ribonuclease before analysis (results not shown), indicating that direct analysis is a good measure of the levels of protein non-covalently associated with the RNA. Figure 2(a) shows the profiles obtained with RNA of bisulphite-treated virus particles. There was a 20-fold increase in the levels of protein associated with ribonuclease-treated RNA compared to RNA analysed directly. This is due to protein covalently cross-linked to the RNA during bisulphite treatment and is equivalent to approx. 3% of the total capsid protein. All four structural proteins, VP1 to 4, were covalently cross-linked to the virus RNA. Estimates of the number of copies of each of the structural proteins cross-linked to the RNA per virus particle, derived from Fig. 2(a), are shown in Table 2.

The bisulphite-mediated cross-linking of proteins to RNA occurs via basic amino acids. Table 2 shows the number of protein molecules cross-linked to the RNA per virus particle per lysine group predicted to be on the internal surface of the 12S particle. This gives an approximate measure of the efficiency of the cross-linking reaction but neglects the contribution of arginine residues to cross-link formation. The extent of cross-linking of VP1 to 3 broadly reflects the predicted number of available lysine residues and the results imply that cross-linking is relatively inefficient. The efficiency of cross-linking of VP4 was at least tenfold higher but cannot be quantified accurately due to the imprecision regarding the number of lysine residues available for cross-linking. However, the results indicate that VP4 may be closely associated with the virus RNA.
Fig. 2(b) shows the profiles obtained with RNA from bisulphite-treated virus particles that were subsequently treated with trypsin. The structural proteins VP2 to 4 are present in amounts similar to those observed in virus particles treated with bisulphite only (Fig. 2a), the difference in d.p.m. observed being due to the difference in specific activity of the $[\text{35S}]$methionine used. Table 2 shows the close agreement between experiments when this difference is taken into account. Both of the fragments generated by trypsin digestion of intact virus particles, VP1(L) and VP1(S) (Burroughs et al., 1971), are cross-linked to the virus RNA in similar amounts (Table 2). It was not possible to judge the efficiency of the cross-linking as the number of lysine groups available for cross-linking could not be determined precisely.

**DISCUSSION**

Laporte & Lenoir (1973) and Talbot et al. (1973) concluded, using lactoperoxidase- or chloramine T-catalysed iodination of tyrosine residues of FMDV, that VP1 was the predominant surface protein, with a much smaller contribution (5%) from VP3. These results are consistent with data from other picornaviruses where tyrosine modification results in the predominant labelling of VP1 in mengovirus (Lund et al., 1977) and poliovirus (Beneke et al., 1977). However, when small, lysine-modifying reagents are used, VP1 to 3 of poliovirus (Wetz & Habermehl, 1979) and rhinovirus (Lonberg-Holm & Butterworth, 1976) are labelled. There appears to be a consistent difference in the results obtained from the two types of modification reaction. In general, it is thought that the results obtained by lysine modification give a better reflection of surface exposure than those obtained from tyrosine modification (see Introduction). It would therefore appear that regions of VP1, VP2 and VP3 are present on the surface of the FMDV particle and, more generally, on the surface of all picornaviruses as the recent X-ray crystallographic studies by Hogle et al. (1985) and Rossmann et al. (1985) with poliovirus and rhinovirus respectively show unequivocally that VP1, VP2 and VP3 of these viruses are partly exposed on the surface of the virus particles.

Assuming that the number of lysine residues modified per unit area is roughly constant, the results indicate that more regions of VP1 than of VP2 or VP3 are exposed on the virus surface. This is compatible with the role of VP1 in cell attachment (Cavanagh et al., 1977) and in the stimulation of the immune response (Laporte et al., 1973).

VP4 does not appear to be modified by $^{125}$I-labelled Bolton & Hunter reagent in the intact virus particle, suggesting an internal location. This view is supported by the findings of Talbot et al. (1973) who observed iodination of VP4 after cleavage of VP1 with trypsin, suggesting that VP4 underlies VP1. The X-ray studies on poliovirus and rhinovirus referred to above (Hogle et al., 1985; Rossmann et al., 1985) provide conclusive evidence that VP4 is entirely internal in these viruses also.

The increased level of modification of 12S particles compared to intact virus particles indicates that there are lysine residues exposed on the internal surface of the 12S particles. These residues are available for interactions with the virus RNA and may, in addition, account for the increased degradation of VP1 and the degradation of VP2 observed when 12S particles, as opposed to virus particles, are digested with trypsin (King & Newman, 1980).

There was substantial degradation of the virus RNA and breakdown of the virus particles at 37 °C, both in the presence or absence of bisulphite. This may be attributed to the activation of the endogenous endoribonuclease of the virus particles (Denoya et al., 1978b) resulting in degradation of the virus RNA and consequent virus breakdown. Since RNA degradation within the virus particle has been correlated with their increased disruption at a lower temperature (Bachrach, 1968; Denoya et al., 1978a), intact RNA may contribute to capsid stability.

The results show that the structural proteins VP1 to 3 can be cross-linked to the virus RNA and thus they have regions both on the surface of the virus and regions in contact with the RNA. VP4 appears to be primarily involved in RNA interactions: it does not react with surface labelling reagents, indicating an internal location and the efficiency of cross-linkage to the RNA is significantly higher than that of the other structural proteins; it may therefore be considered as the 'core' protein of FMDV.

Nucleic acid-binding regions of proteins appear to have common features, namely a high
content of basic amino acids, a significant number of acidic amino acids and pairs of adjacent basic and acidic residues (Ehresmann et al., 1980). In addition, clusters of basic and acidic amino acids with proline residues appear to be involved in nucleic acid-binding (Garoff et al., 1980). Analysis of the amino acid sequences of VP1, VP2 and VP3 (Boothroyd et al., 1982) shows that they contain a number of such regions which could be implicated in the interactions of these proteins with the RNA. In contrast, VP4 contains only one lysine residue, has a blocked amino terminus and does not contain any regions of the type described. However, amino acids 1 to 30 of VP4 contain a high proportion (37\%) of glutamine and asparagine residues which may form hydrogen bonds with the phosphate groups of the RNA and amino acids 1 to 17 of VP4 contain a very high proportion (65\%) of helix-breaking amino acids (Chou & Fasman, 1974) which may be capable of intimate interaction with the RNA due to the high flexibility of the polypeptide chain.

Wetz & Habermehl (1982) have shown that VP4 of acid-stable poliovirus can be covalently linked to the RNA during u.v. irradiation, suggesting that VP4 may have a ‘core’ role in poliovirus. However, the amino acid sequence of poliovirus VP4 (Nomoto et al., 1982) is strikingly different from that of FMDV VP4: poliovirus VP4 is basic and it does not have regions with high proportions of helix-breaking amino acids or amide-containing amino acid residues. However, it does contain the clusters of charged amino acids predicted to be nucleic acid-binding sites. This difference may be a factor in the different forces stabilizing the two viruses below pH 7 and it is possibly relevant that VP4 (\alpha) of the acid-labile encephalomyocarditis virus, like that of FMDV, is acidic and has a high proportion of helix-breaking and amide-containing amino acids (Palmenberg et al., 1984).

The picornaviruses appear to be stabilized by a combination of protein–protein and RNA–protein interactions, but the relative contributions of the interactions appear to vary within the group. In poliovirus the stabilizing forces seem to involve largely, but not exclusively, protein–protein interactions between the major subunits of the capsid. This also appears to be the case in FMDV ‘natural empty’ particles, RNA-free capsids which contain 60 copies of VP1, VP3 and VP0 (the precursor of VP2 and VP4) (Rowlands et al., 1975). However, after encapsidation of the RNA and concurrent cleavage of VP0 to VP2 and VP4, RNA–protein interactions appear to contribute to the stability of the FMDV capsid and protein–protein interactions are weakened. Breakdown to 12S particles, rather than ‘artificial empty’ particles, now occurs on destabilization. The changes in stabilizing interactions may be due to the transfer of some of the interactions of VP2 and VP4 from the other structural proteins to the virus RNA.

Two regions of FMDV VP1, amino acids 140 to 160 and 200 to 213, have been shown to stimulate the production of neutralizing antibodies (Strohmaier et al., 1982; Bittle et al., 1982) and these regions may be assumed to be on the surface of the virus particle. Trypsin cleaves FMDV VP1 at, or near, the first of these regions to produce the VP1(L) and VP1(S) fragments. Therefore, the interactions observed between the VP1(S) fragment and the virus RNA must occur in a region between the two sites capable of stimulating the production of neutralizing antibodies. Two explanations can be put forward: either the RNA passes through the capsid to react with the interacting regions near the surface of the virus particle or the interacting region of VP1 passes through the capsid to interact with the RNA within the virus particle. Neutron scattering studies (Jacrot et al., 1977) and X-ray diffraction analysis (Harrison et al., 1980; Hogle et al., 1985; Rossmann et al., 1985) have shown that the protein and nucleic acid regions of virus capsids tend to be discrete. Furthermore, hydrophilicity analysis by the method of Hopp & Woods (1981) of the amino acid sequence of FMDV VP1 has shown that there are very hydrophobic regions of the protein between the two antigenic regions, compatible with the passage of VP1 across the protein core of the capsid. The length of these regions, about seven amino acids, is sufficient to cross the capsid which has been shown to be approximately 4 nm in width (Vasquez et al., 1979). In the hydrophobic regions is a cluster of charged amino acids (at amino acids 176 to 179) which may be proposed as an RNA-binding site. These data suggest that the second explanation proposed above is more likely.

The iodination of tyrosine residues of VP4 after in situ cleavage of VP1 by trypsin (Talbot et al., 1973) suggests that VP4 underlies VP1 near the site of the interaction between VP1 and the
virus RNA. From this observation and the discussion above, a model can be proposed for the virus capsid in the proximity of the major antigenic determinants of VP1 (Fig. 3). The model has a number of features: during production of 12S particles or 'artificial empty' capsids the disruption of the proposed interactions of the RNA and/or VP4 with a region of VP1 in close proximity to the antigenic regions of the protein may cause changes in its antigenic determinants, resulting in the different antigenicity of these particles (Rowlands et al., 1975; Cartwright et al., 1982).

The model also proposes that the two antigenic regions of VP1 are close together, possibly combining to form a single antigenic region. This proposal is supported by recent evidence that a peptide corresponding to amino acids 141 to 160 and 200 to 213 joined together is more active than either of the sequences alone in blocking the reaction of virus particles with a neutralizing monoclonal antibody and in eliciting neutralizing antibody (Parry et al., 1985; DiMarchi et al., 1986).

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