Model of the equine rhinitis A virus capsid: identification of a major neutralizing immunogenic site

Gernot Kriegshäuser,1† Gordana Wutz,1 Susan Lea,2 David Stuart,2‡ Tim Skern1 and Ernst Kuechler1

1Max F. Perutz Laboratories, University Departments at the Vienna Biocenter, Department of Medical Biochemistry, Division of Biochemistry, University of Vienna, Dr Bohr Gasse 9/3, A-1030 Vienna, Austria
2Laboratory of Molecular Biophysics, Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, UK

Correspondence
Ernst Kuechler
kuechler@bch.univie.ac.at

Received 14 March 2003
Accepted 21 May 2003

Mouse monoclonal antibodies (mAbs) were employed to select neutralization escape mutants of equine rhinitis A virus (ERAV). Amino acid changes in the ERAV mutants resulting in resistance to neutralization were identified in capsid protein VP1 at Lys-114, Pro-240 and Thr-241. Although the changes were located in different parts of the polypeptide chain, these mutants exhibited cross-resistance against all four mAbs employed, indicating that these residues contribute to a single immunogenic site. To explain this result, we constructed a model of the three-dimensional structure of the ERAV capsid based on comparison with the closely related foot-and-mouth disease virus (FMDV O1). According to this model, VP1 is folded so that Lys-114 is in the βE–βF loop of the polypeptide chain at a considerable distance from Pro-240 and Trp-241 in the C-terminal region. However, around the fivefold axis of symmetry, the C terminus of VP1 in each protomer extends to the βE–βF loop of the adjacent VP1 in the next protomer. We therefore propose that the immunogenic site in ERAV is formed as a result of the close proximity of the Lys-114 residue in the βE–βF loop of one VP1 molecule and of the Pro-240/Thr-241 residues in the adjacent VP1 polypeptide chain. In terms of the overall architecture of the viral capsid structure, this site in ERAV most closely resembles the immunogenic site 1 of FMDV O1.

INTRODUCTION

Equine rhinitis A virus (ERAV) infection of horses occurs worldwide and is recognized as a cause of acute respiratory disease with symptoms such as fever, nasal discharge, coughing and pharyngitis (Ditchfield & MacPherson, 1965; Hofer et al., 1973; Holmes et al., 1978; Plummer, 1962; Studdert & Gleeson, 1977). ERAV belongs to the family of Picornaviridae. Like all picornaviruses, it has an icosahedral capsid comprising 60 copies each of proteins VP1, VP2, VP3 and VP4. The virion has a dimension of 25–30 nm and contains a single-stranded RNA of positive polarity of approximately 8000 nucleotides (Newman et al., 1977).

Immunologically, four serotypes of equine rhinitis viruses (ERVs) have been identified (Fukunaga et al., 1983; Steck et al., 1978). ERAV and the only two representatives of the genus Erbovirus known so far, equine rhinitis B virus (ERBV) type 1 and type 2, are acid-labile, whereas ERV serotype 4 (which has only been classified serologically) is comparatively stable following treatment at pH lower than 5. ERAV is the most frequent ERV serotype in Europe. In some horse populations, more than half of the horses of 1 year of age or older have high titres of neutralizing antibody against ERAV, indicating that the virus is widespread (Studdert & Gleeson, 1978). The disease caused by ERAV resembles the common cold in man brought about by human rhinoviruses (Li et al., 1997; McCollum & Timoney, 1991; Newman et al., 1977). However, the presence of a viraemia during infection, the occurrence of persistent infections, the long-term faecal and urinary shedding of virus particles, the broad host range in vitro and in vivo, the lack of extensive antigenic variation and the physicochemical properties of ERAV are more reminiscent of foot-and-mouth disease virus (FMDV) (Newman et al., 1973). Indeed, sequencing studies have demonstrated that ERAV is closely related to FMDV (Li et al., 1996; Wutz et al., 1996), one of the most contagious animal pathogens. Due to the extensive sequence homology and similarity in the order of...
proteins on the genome, ERAV has been placed in the genus *Aphthovirus* with FMDV. Sites in the capsid of the FMDV virion that elicit a neutralizing antibody response have been identified (Mateu, 1995). The localization of such sites is an important prerequisite for the understanding of virus immunogenicity and the mechanism of neutralization by antibodies.

ERAV represents the first aphthovirus of low to moderate pathogenicity. ERAV may thus serve as a model virus for studying aphthovirus biology. It was therefore of interest to characterize the immunogenic epitopes of ERAV and to compare their location within the viral capsid with that of FMDV. An experimental approach to define epitopes is to select mutant viruses that are able to escape neutralization by monoclonal antibodies (mAbs) followed by identification of the amino acids altered in the escape mutants. We have investigated nine independently isolated ERAV escape mutants selected against a set of four neutralizing mAbs. All mutants showed extensive cross-resistance when tested against the other mAbs, indicating that they belong to the same immunogenic site. The corresponding amino acid changes were found to occur exclusively in VP1. The sites of the mutations were mapped on a model of the three-dimensional structure of ERAV based on homology with the known FMDV structure (Acharya et al., 1989). The data support a model of the ERAV capsid in which a major neutralizing immunogenic site is formed by amino acid residues from two adjacent VP1 molecules.

**METHODS**

**Virus, cells and media.** ERAV (strain PERV) (Plummer, 1962), as well as a reference antisera, was originally obtained from M. Weiss, Institute of Virology, University of Berne, Switzerland. ERAV was plaque-purified, propagated in rabbit kidney cells (RK-13) and centrifuged on sucrose or cesium chloride gradients (Brown & Cartwright, 1963; Newman et al., 1973). The identity of the virus was checked by immunoprecipitation with the reference antiserum. RK-13 cells were routinely grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% foetal calf serum (FCS). Ag563B RK-13 cells were propagated in RPMI 1640 medium supplemented with 20% FCS. Hybridoma cells were selected in HAT medium, and allowed to attach for 1 h at 37°C. The wells were washed twice with 2 ml PBS and overlaid with 0.8% agar in a 1:1 (v/v) mixture of DMEM and the hybridoma supernatant, followed by an incubation period of 2–3 days at 37°C. Mutant plaques were picked and freeze-thawed twice in 1 ml DMEM without FCS. All variants were purified by replaques twice in the presence of the antibody. Frequencies of mAb-resistant mutants were determined for each mAb by a plaque reduction assay. The frequencies were calculated as the average number of plaques in the presence of mAb divided by the number of p.f.u. plated.

**Virus neutralization test.** Virus neutralization was carried out in 96-well tissue culture plates. Fifty μl of each serum was diluted in 50 μl DMEM and serially diluted twofold to a dilution of 1:512. Fifty μl ERAV was added to each well in a 100-fold dilution of the TCID50 ml−1. Following incubation at 37°C for 1 h, 10 μl freshly turgid PK-13 cells in DMEM containing 2% FCS was added to each well. Plates were incubated at 37°C for another 36–48 h. At this time, the cytopathic effect of the virus control lacking the serum reached 100 TCID50. Neutralization titres greater than 1–2 were regarded as positive.

**RT-PCR and DNA sequencing.** Viral RNA was isolated from each of the nine mAb-resistant mutants by mixing 300 μl TRizol reagent (Gibco BRL) with 100 μl tissue culture supernatant containing virus at ~102 TCID50 ml−1. After denaturation, 80 μl chloroform was added. The mixture was vigorously shaken, incubated for 10 min on ice, centrifuged and the viral RNA was precipitated from the aqueous phase with 200 μl isopropanol. After centrifugation, the RNA pellet was washed with 500 μl 75% ethanol and recentrifuged. Finally, the RNA pellet was briefly air dried and resuspended in 11 μl RNase-free water. Reverse transcription of viral RNA was performed with primer P2 (see below). RNA and 20 pmol of primer were mixed, incubated for 10 min at 60°C and cooled at room temperature. First-strand cDNA was synthesized by addition of 18 U AMV reverse transcriptase (Promega) in the presence of each dNTP at 1 mM and 20 U human placental RNase inhibitor (Promega), in a reaction volume of 18 μl. Primer pairs P1/P2 (5′-TGATTCTATG-CTTNTGGTG-3′/5′-CTCRACRTCTCCMGCCAA-3′), P3/P5 (5′-CATGATCTGATGTC-3′/5′-GGCGTGGTTCTGCTGTCTC-3′) and P5/P6 (5′-GGCITGTTCTTGCTTAG-3′/5′-GACATGAAGAATCTGTG-3′) were synthesized to generate three overlapping PCR fragments encoding virus capsid proteins VP1, VP3 and VP2, respectively. ERAV cDNA was amplified in 10 μl by PCR over 40 cycles, each consisting of 0.5 min at 94°C, 1 min at 55°C and 1.5 min at 72°C in a total volume of 50 μl. The solution was adjusted to a final concentration of 125 μM dATP, dCTP, dTTP and dGTP, 1.5 U Pfu polymerase (Stratagene), 0.15 μM of each primer and 2.5 μl Pfu polymerase (Stratagene). PCR amplification products were subjected to agarose gel electrophoresis and the specific band was excised and extracted with phenol/chloroform. DNA sequencing was performed once on each fragment using an ALF sequencer (Pharmacia).

**Modelling of the ERAV capsid structure.** To determine the secondary structure elements in the ERAV capsid proteins, the ERAV protein sequence was aligned with those of FMDV O1BFS, mengovirus and human rhinovirus (HRV) 14. The three-dimensional structures of these viruses are known. The putative positions of α-helices and β-strands as well as the lengths of the loop structures were determined. The ERAV capsid protein sequences share a high level of sequence similarity with the capsid proteins of
FMDV O₁ (VP1 to VP3 between 33 and 39 %, VP4 59 %). A model of ERAV was therefore constructed based on the structure of FMDV O₁ (PDB1FOD.ENT). For the core of the structure for which the length of proteins did not vary between FMDV and ERAV, the model was constructed by simple substitution of the FMDV side chains for those present in ERAV (using the program Calpha; R. Esnouf, Structural Biology Division, University of Oxford, UK). Insertions and deletions were modelled using the loop structures from other picornaviruses (mengovirus or HRV14, selected according to the level of sequence homology) using the program O (Jones et al., 1991) on an Evans and Sutherland PS390 computer graphics system. Details such as the location of the side chains were not considered during the modelling process, other than that they were all built in the most common rotamer for each side chain. The pentameric subunit was reconstructed from the protomer model using the Symmetrias program [written by I. Fita, Centre d’Investigacio i Desenvolupament (CSIS), Barcelona, Spain.]

RESULTS

Selection and frequency of antibody-resistant ERAV mutants

Four mouse hybridoma lines (designated 1, 2, 3 and 4) were generated that secreted mAbs capable of neutralizing ERAV. Each of these mAbs was employed to select viral mutants resistant to neutralization (escape mutants). For determination of the frequency of mutation, ERAV propagated at a low m.o.i. (0·1 p.f.u. per cell) was incubated with each of the mAbs in a plaque reduction assay. Mutants were obtained at 6·25 × 10⁻⁴ for mAb1, 5·25 × 10⁻⁴ for mAb2, 4·0 × 10⁻⁴ for mAb3 and 4·3 × 10⁻⁴ for mAb4, respectively; values represent the average of three separate experiments. The rates were comparable with those obtained from other picornaviruses, which exhibit mutation frequencies to mAb antibodies ranging from 10⁻³ to 10⁻⁷ (Boege et al., 1991; Lea et al., 1994; Sherry et al., 1985).

Antibody resistance patterns define a single neutralization epitope

ERAV mutants were selected against the four mAbs. Mutants were tested with each of the four mAbs in order to define the characteristic neutralization pattern. As expected, the ERAV mutants exhibited resistance against the mAb used for neutralization-escape selection. However, all mutants also showed resistance to the other three mAbs, indicating that there was extensive cross-neutralization. This indicated that all four mAbs employed bound either to the same or to different epitope(s) within a single neutralizing immunogenic site. When the mAbs were tested in a Western blot, no reactivity to ERAV capsid proteins VP1, VP2 and VP3 was observed (data not shown). This was taken as a first indication that the epitopes recognized by the mAbs were discontinuous and that the neutralizing antigenic site might be generated by the folding of the polypeptide chain(s) in the ERAV capsid. All escape mutants were still neutralized by rabbit polyclonal antisera raised against ERAV, indicating that other antigenic sites are present.

Identification of mutations in the genome of ERAV escape mutants

In order to map the mutations in the ERAV genome, RNA was isolated from each of nine independently isolated mutant viruses and subjected to RT-PCR. The PCR was designed to amplify a 1038 bp region comprising all of VP1, a 766 bp region containing the complete VP3 and a 778 bp region spanning VP2. As VP4 is not exposed on the surface of picornaviruses, this region was not examined for mutations. PCR products were extracted from the agarose gel and sequenced by the dideoxy chain termination method. Table 1 summarizes the nucleotide and amino acid changes obtained in the escape mutants. All mutations occurred in VP1; no changes were found in capsid proteins VP2 or VP3. Surprisingly, only three different sites were detected, corresponding to amino acids in positions 114, 240 and 241 of VP1. According to the Rossmann system, positions in the viral capsid are indicated as 1114, 1240 and 1241, respectively, with the first digit representing VP1. In mutant 1/1, Lys-1114 was substituted by Arg. Mutant 2/1 contained the substitution Pro-1240→Leu, whereas mutants 1/2, 2/2, 3/1, 3/2 and 4/1 had the replacement Thr-1241→Pro. Two mutant viruses (1/3 and 2/3) contained both mutations Lys-1114→Arg and Thr-1241→Pro. None of the amino acid alterations resulted in a change of the amino acid charge.

As the same mutations were identified in several independent isolates, the corresponding epitopes obviously represent major immunogenic determinants in the ERAV capsid. However, since the mAbs exhibited extensive cross-reaction, the epitopes must be part of a common neutralizing antigenic site. Lys-1114→Arg and Thr-1241→Pro occurred

Table 1. Changes in nucleotide sequence and amino acid sequence for the external capsid protein VP1

<table>
<thead>
<tr>
<th>Mutant virus</th>
<th>Nucleotide changes*</th>
<th>Amino acid changes in VP1†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/1</td>
<td>3458 A→G</td>
<td>1114 K→R</td>
</tr>
<tr>
<td>1/2</td>
<td>3838 A→C</td>
<td>1241 T→P</td>
</tr>
<tr>
<td>1/3</td>
<td>3458 A→G</td>
<td>1114 K→R</td>
</tr>
<tr>
<td>2/1</td>
<td>3836 C→T</td>
<td>1241 T→P</td>
</tr>
<tr>
<td>2/2</td>
<td>3838 A→C</td>
<td>1241 T→P</td>
</tr>
<tr>
<td>2/3</td>
<td>3458 A→G</td>
<td>1114 K→R</td>
</tr>
<tr>
<td>3/1</td>
<td>3838 A→C</td>
<td>1241 T→P</td>
</tr>
<tr>
<td>3/2</td>
<td>3838 A→C</td>
<td>1241 T→P</td>
</tr>
<tr>
<td>4/1</td>
<td>3838 A→C</td>
<td>1241 T→P</td>
</tr>
</tbody>
</table>

*The nucleotide numbers refer to the L fragment of the ERAV genome (Wutz et al., 1996).
†The amino acid residues are identified according to the Rossmann system, with the first digit representing viral capsid protein VP1, VP2 or VP3 and the next three digits indicating position from the N terminus.
both as individual mutations and together as a double mutation. Apparently, in the virus, these residues are close enough to contribute to a common neutralizing antigenic site, despite the fact that they are more than a hundred amino acids apart in the primary sequence of VP1. In order to understand how Lys-1114, Pro-1240 and Thr-1241 are

![Diagram of secondary structure elements of ERAV VP1 compared to FMDV, mengovirus, and HRV14](image)

**Fig. 1.** Position of escape mutants in VP1 of ERAV. The predicted secondary structure elements of ERAV VP1 are shown, together with those of FMDV, mengovirus, and HRV14, as determined by X-ray crystallography. Identical amino acids that match with at least two other sequences are in bold. The amino acid changes found in the escape mutants (Lys-1114 $\rightarrow$ Arg, Pro-1240 $\rightarrow$ Leu and Thr-1241 $\rightarrow$ Pro) are shown above the ERAV sequence (adapted from Wutz et al., 1996).
brought into close proximity by the folding and arrangement of the VP1 polypeptide chains in the ERAV pentamer, we developed a model of the ERAV capsid structure.

A structural model of the ERAV pentamer

Based on the known three-dimensional structures of the capsids of FMDV (Acharya et al., 1989; Logan et al., 1993), mengovirus (Luo et al., 1987) and HRV14 (Rossmann et al., 1985), structural alignments of the three capsid proteins of ERAV were produced. Using the known secondary structures as templates, predictions of the positions of secondary structure elements and of the lengths of loop regions of ERAV were made (Wutz et al., 1996). Fig. 1 shows that the Pro-1240→Leu and Thr-1241→Pro substitutions, the two predominant mutations leading to mAb resistance, were located five and four amino acids from the C terminus of VP1, respectively. In several picornaviruses, i.e. FMDV, HRV2, HRV14 and poliovirus type 3, C-terminal sequences are found to be involved in antigenic sites (Mateu, 1995). Indeed, the 17 C-terminal residues of VP1 of FMDV form a long arm that resides on the surface of the capsid (Acharya et al., 1989), thus being accessible to antibodies (reviewed in Rowlands & Brown, 2002). The third amino acid alteration detected, which involves the Lys-1114→Arg change, was located in the central part of the polypeptide chain in the predicted βE–βF loop of VP1 (Fig. 1). This loop is predicted to be longer in ERAV than in FMDV. As this loop is located on the external surface of the capsid, the observed extension in ERAV might improve accessibility by antibodies and thus increase the antigenicity of the βE–βF loop. Notably, this loop has never been found to be antigenic in FMDV. However, in poliovirus serotypes 2 and 3, the βE–βF loop has been reported to harbour an antigenic site (Mateu, 1995).

Nevertheless, this prediction does not explain how mutations in the βE–βF loop and the C terminus can all be part of the same antigenic site. To investigate this, we constructed a three-dimensional model of the ERAV pentamer. By integrating alignments of picornavirus capsid proteins and three-dimensional structures of several picornaviruses, a model of the ERAV protomer was first generated. The protein sequences of the ERAV VP1, VP2 and VP3 proteins were compared with the FMDV O1 BFS, mengovirus and HRV14 proteins of known three-dimensional structure having similar amino acid sequences. From the alignment, it was possible to determine the secondary structure elements in the ERAV capsid proteins. The positions of insertions and deletions were moved to optimize the structural correspondence when comparing picornavirus capsids. Since the amino acid sequence of the ERAV capsid proteins was most similar to that of the FMDV capsid proteins (Wutz et al., 1996), the ERAV protein sequence was mapped on to FMDV wherever possible simply by replacing one kind of residue for another. Such replacements affected the course of the main chain only minimally. The loop regions containing insertions or deletions were modelled according to the equivalent loops in mengovirus or HRV14. The final model of the ERAV protomer together with all insertions relative to FMDV is shown in Fig. 2. The main differences relative to FMDV were observed in the VP1 protein where the loops in ERAV were generally longer; a notable exception, however, was the βG–βH loop (the FMDV main antigenic site), which was 13 amino acids shorter in ERAV. The βD–βE, βE–βG1, βH–βI and βE–βF loops of VP1 of ERAV were close to the fivefold axis of symmetry, which is not the case in FMDV. In their length and probably their tertiary structure, they were more similar to mengovirus and HRV14. The protein sequences of VP2 and VP3 are much more conserved between ERAV and FMDV than is the case for VP1. In ERAV VP2, there is essentially only one major insertion, in the βE–zB loop. ERAV VP3 contains a single insertion in the βH–βI loop.

Fig. 2 predicts that the βE–βF loop and the C terminus of VP1 of ERAV are both exposed on the surface and should therefore constitute potential antigenic sites, as was indeed demonstrated by the amino acid changes in the neutralization-resistant virus mutants. The data presented suggest a single immunogenic site recognized by all four neutralizing mAbs, which is formed by amino acid residues in the βE–βF loop and in the C-terminal region of VP1. Surprisingly, in the model of the ERAV protomer, the βE–βF loop and the C terminus of the VP1 polypeptide chain are located at a considerable distance from each other. However, it was considered possible that the arrangement of the polypeptide chains in the pentamer would bring the two regions bearing the escape mutations into close proximity.

Thus, a pentameric subunit was constructed from the protomer model (Fig. 3). The ERAV model placed the βD–βE and the βE–βF loops of VP1 around the fivefold axis of symmetry. The lengths of these loops were similar to those of HRV14 and mengovirus in which these loops are located in close proximity. In contrast, FMDV lacks the loops clustering around the fivefold axis. In addition, Fig. 3 shows that the C-terminal residues of VP1 formed a long extended arm, which was exposed on the surface. This arm ran in a clockwise fashion around the fivefold axis of symmetry from VP1 in one protomer over VP3 until it nestled against the βE–βF loop of the adjacent VP1 of the next protomer. A similar situation is encountered in FMDV, except that the βE–βF loop is much shorter than in ERAV (Acharya et al., 1989). It is therefore conceivable that amino acids in the βE–βF loop of ERAV may contribute to the immunogenic site. According to the ERAV model, the distance between the ε-carbon atom of Lys-1114 in the βE–βF loop of one VP1 molecule and the ε-carbon atom of Pro-1240 in the C-terminal region of the adjacent VP1 polypeptide chain is around 7.8 Å. Thus, the side chains of the amino acids in the βE–βF loop and in the C-terminal region of two adjacent VP1 molecules may be close enough to form a common immunogenic site in the ERAV capsid and therefore explain the immunological data.
DISCUSSION

The similarities between ERAV and FMDV in genomic structure, nucleotide sequence and physico-chemical properties have resulted in ERAV being included in the Aphthovirus genus of the Picornaviridae family. Despite the wealth of information on the antigenic structure of FMDV, little is known about the antigenicity of ERAV, except that, not surprisingly, the capsid protein VP1 is involved (Warner et al., 2001).

To investigate ERAV antigenicity, we set out to generate neutralizing mAbs from infectious wild-type ERAV (strain PERV). In a previous report, mAbs were generated against UV-inactivated ERAV (Varrasso et al., 2001); however, none of these mAbs was able to neutralize infectious ERAV. In contrast, in this study, we obtained only four mAbs, all of which were capable of neutralizing ERAV. In contrast, in this study, we obtained only four mAbs, all of which were capable of neutralizing ERAV. Subsequently, incubation of ERAV with these mAbs, a total of nine escape mutants was selected. Nucleotide sequencing of PCR fragments generated from these mutants revealed that three characteristic mutations (see Table 1) were responsible for allowing ERAV to escape neutralization by any of the mAbs. The ability of the mutants to escape neutralization by all mAbs indicated the presence of only one antigenic site. The distribution of the three mutations giving rise to escape mutants demonstrated that the epitope was a structural one.

The mutation Lys-1114→Arg lies in an exposed region of VP1 in the βE–βF loop. Interestingly, this proposed loop was shown to be the most diverse when ten independent ERAV sequences were compared, although the Lys-1114→Arg mutation was not itself observed (Varrasso et al., 2001). In contrast, no difference between the ERAV sequences was found in the region of amino acids Pro-1240 and Thr-1241.

To explain the structural nature of this ERAV epitope in the absence of the three-dimensional structure of ERAV, we set out to build a model of the ERAV capsid, based on known picornavirus structures, notably that of FMDV. The positions of the backbone atoms of the polypeptide chains of ERAV VP1, VP2 and VP3 could be modelled with some accuracy. The regions in all three capsid proteins of ERAV corresponding to the β-strands appeared to be structurally similar to those in FMDV. Significantly, however, the lengths of several loops were different between the capsid proteins of the two viruses. One of the most prominent differences was the length of the loop βE–βF; this loop contains one of the escape mutations as well as many of the differences between
Fig. 3. Pentameric association of ERAV protomers. (A) Orthogonal view of the virtual bonds joining α-carbon atoms. The view of the fivefold axis is from the outside. Colour coding of the capsid proteins is the same as in Fig. 2 except that only the βE–βF loop is depicted in yellow and the C-terminal portion (17 amino acid residues) of VP1 is depicted in white. (B) Side view of the pentamer. The outer surface is at the top. The βE–βF loop and the C-terminal portion (17 amino acid residues) of VP1 are depicted in yellow and pink, respectively. Amino acid residues that were found altered in virus mutants are indicated.
the ten independent strains examined by Varrasso et al. (2001). In contrast, in FMDV, the main antigenic site is located in the βG–βH loop. This loop was predicted to be much shorter in ERAV.

Computer assembly of the predicted ERAV protomer into pentamers provided an explanation of how mutations lying over 100 amino acids apart in the primary sequence could be part of the same antigenic site. ERAV VP1 is predicted to have a long C-terminal extension, which reaches across the adjacent protomer, so that the distance between the χ-carbon atoms of Lys-1114 and Pro-1240 is less than 8 Å. Thus, the side-chains of Lys-1114, Pro-1240 and Thr-1241 could easily be recognized by the same antibody molecule.

The antigenic site identified here in ERAV is clearly analogous to antigenic site 1 of FMDV serotype O1 (Rowlands & Brown, 2002). In both cases, the sites are formed by contributions from a loop in VP1 (loop βE–βF in ERAV and loop βG–βH in FMDV) and from amino acids from the C terminus. Thus, although the antigenicities of the viruses are clearly different, the similar architecture of an important antigenic site represents a further illustration of the relationship between the two viruses and provides support for their inclusion in the same genus.

ACKNOWLEDGEMENTS

This work was supported by grants from the Austrian National Bank (Jubiläumsfonds Nr 7140) to E. K. and from the Austrian Science Foundation (WK-01) to G. K. We thank M. Weiss (University of Berne, Switzerland) and N. Nowotny (Veterinary University, Vienna) for ERAV and for polyclonal rabbit antiserum. S. M. L. was supported by a Royal Society Dorothy Hodgkin Research Fellowship.

REFERENCES


