Synthetic peptides mimic subtype specificity of foot-and-mouth disease virus

B.E. Clarke, A.R. Carroll, D.J. Rowlands, B.H. Nicholson+, R.A. Houghten*, R.A. Lerner* and F. Brown

Biochemistry Department, Animal Virus Research Institute, Pirbright, Woking, Surrey GU24 ONF, +Department of Physiology and Biochemistry, University of Reading, England and **Research Institute of Scripps Clinic, La Jolla, CA 92037, USA*

Received 4 May 1983

The major immunogen of foot-and-mouth disease virus (FMDV) is located between amino acids 141–160 of the capsid protein VP1. Synthetic peptides corresponding to the major immunogenic region give good neutralising antibody responses and protection in guinea pigs. To define more precisely the immunogenic site of the virus, we have examined serological differences between subtypes of the A serotype using synthetic peptides covering the 141–160 region. We show that these synthetic peptides carry determinants which mimic the subtype specificity of the virus. The correlation between these results and predictive structural models, based on the amino acid sequence, is discussed.

Picornavirus *Foot-and-mouth disease virus* *Antigenic variation* *Synthetic peptide* *Structural model*

1. INTRODUCTION

Foot-and-mouth disease, a highly contagious disease of cloven-hooved animals, is of considerable economic importance. The causative agent is a picornavirus having a single-stranded positive sense RNA genome of about 8000 nucleotides [1]. Foot-and-mouth disease virus (FMDV) occurs as 7 distinct serotypes and infection with one serotype does not confer immunity against any of the others. Within each serotype further antigenic differences occur and at least 60 subtypes of the 7 serotypes have been identified. These differences are frequently great enough to reduce considerably the degree of cross-protection conferred by one subtype against another within the same serotype [2]. This poses a major problem in vaccination programmes. It is therefore of considerable importance to define precisely the nature of the major immunogenic site, so that structural features which determine serotype and subtype specificity may be better understood.

The major immunogenic region is located on the viral capsid protein, VP1 [3] and, from the use of defined chemical and enzymatic fragments of this protein [4], it has been predicted that amino acid sequences 146–154 and 200–213 contain immunogenic sites. The advent of recombinant DNA technology has allowed the sequencing of these regions in several serotypes of the virus [5–7]. The region 141–160 has been shown to be highly variable and hydrophilic in character, suggesting that it is a major antigenic site. This has been further supported by the observations that synthetic peptides corresponding to this region give good neutralising antibody responses and protection from virus challenge in guinea pigs [8,9]. Synthetic peptides corresponding to region 200–213 also elicit neuralizing antibody, although at a much

Nomenclature: Numbering of the amino acids of VP1 is normalised to that of serotype O, sub-type 1 (Kaufbeuren) [7] because of frequent deletions and insertions of amino acids in other virus strains

Published by Elsevier Science Publishers B.V.
00145793/83/$ 3.00

lower level [8]. This work describes an investigation into the nature of the antigenic determinants located in the 141–160 region.

2. MATERIALS AND METHODS

2.1. *Viruses*

Two viruses belonging to subtypes 10 and 12 of the A subtype were used, namely strain 61 and strain 119. These are referred to as A10 and A12, respectively.

2.2. *Preparation of antisera*

Guinea pigs were injected with: (a) virus harvests, grown in baby hamster kidney cells, after inactivating them with 0.05% acetylethyleneimine at 37°C for 6 h; or (b) synthetic peptides covalently linked to keyhole limpet haemocyanin (KLH). In each instance the preparations were first mixed with an equal volume of $Al(OH)_3$ gel. Serum samples were obtained from blood taken 21 days after injection. Antisera were also produced in rabbits by multiple injections of the KLH-coupled peptides according to the schedule described [8].

2.3. *Neutralisation tests*

Ten-fold dilutions of the two viruses were mixed with an equal volume of normal serum or antiserum dilution and 0.03 ml of the mixtures injected into groups of 7-day-old mice. The difference between the titres were taken as the neutralising activity of 0.015 ml of the antiserum dilution.

2.4. *DNA sequencing*

Primer extension experiments were performed basically as in [10] using a synthetic DNA primer kindly supplied by Dr M.J. Gait (Laboratory of Molecular Biology, MRC, Cambridge). Hybridisations were carried out using 20 μg FMDV-RNA and a 5-fold molar excess of radio-labelled primer. After the synthesis of cDNA using reverse transcriptase, standard sequencing reactions were performed [13].

2.5. *Peptide synthesis*

All peptide syntheses and coupling reactions were as in [8]. Essentially peptides were synthesised by a solid-phase method and coupled to a protein carrier (KLH) through a cysteine residue at the C-terminus of the peptide.

3. RESULTS AND DISCUSSION

In previous work, the serotype specificity of the antibodies produced in rabbits by peptides spanning regions 141–160 and 200–213 from serotype O subtype 1, was found to be similar to those elicited by injection of whole virus [8]. This infers that both peptides carry antigenic determinants involved in serotype specificity. To examine those determinants involved in subtype specificity, we performed similar experiments using synthetic peptides corresponding to two virus subtypes from serotype A. We initially checked the amino acid sequence of the viruses being used in this study by a rapid sequencing method [5,10]. This was done because the published sequences were obtained from molecular cloning experiments with the attendant risk that a minor component of the virus population may have been selected (Rowlands et al., in preparation). The nucleotide sequences ob-

A10	ASP GLY THR ASN LYS TYR SER ALA SER ASP	131-140
A12	ASN GLY THR ASN LYS TYR SER ALA SER GLY	
A10	SER - ARG SER - GLY ASP LEU GLY SER	141-150
A12	SER GLY - VAL ARG GLY ASP PHE GLY SER	
A10	ILE ALA ALA ARG VAL ALA THR GLN LEU PRO	151-160
A12	LEU ALA PRO ARG VAL ALA ARG GLN LEU PRO	
A10	ALA SER PHE ASN TYR GLY ALA ILE GLN ALA	161-170
A12	ALA SER PHE ASN TYR GLY ALA ILE LYS ALA	
A10	GLN ALA ILE HIS GLU LEU LEU VAL ARG MET	171-180
A12	GLU THR ILE HIS GLU LEU LEU VAL ARG MET	
A10	LYS ARG ALA GLU LEU TYR CYS PRO LYS PRO	181-190
A12	LYS ARG ALA GLU LEU TYR CYS PRO ARG PRO	
	198A*	
A10	LEU LEU ALA ILE LYS VAL THR SER(GLN)ASP	191-199
A12	LEU LEU ALA ILE GLU VAL SER SER(GLN)ASP	
A10	ARG TYR LYS GLN LYS ILE ILE ALA PRO ALA LYS GLN LEU LEU	200-213
A12	ARG HIS LYS GLN LYS ILE ILE ALA PRO GLY LYS GLN LEU LEU	

Fig.1. Amino acid sequence of the C-terminal region of FMDV strains A10 (61) and A12 (119). Sequences were derived by a primer extension method. Amino acids 141–160 (underlined) indicate the region for which peptides were synthesised. The additional amino acid at position 212 is indicated. *See footnote on title page.

tained are shown in fig.1. The sequence of A10 agrees exactly with that obtained by molecular cloning methods, while that of A12 differs only in the presence of an additional leucine residue at position 212. Nucleotide sequence data now available for 6 of the 7 serotypes (Clarke et al., in preparation) [5–7,11,12] show that the presence of Leu–Leu or Thr–Leu at positions 212–213 is universal. We therefore assume the absence of the leucine at 212 in the cloned A12 [6] may be an artefact of cloning or sequencing. Comparative sequence data between subtypes of serotype A [5,6,11,12] show a good degree of conservation between amino acids 200–213, whereas region 141–160 shows considerable variability. It seems logical therefore that subtype determinants may lie in the latter region.

Table 1

Cross-neutralization of viruses from subtypes A10 and A12 with antivirion and antipeptide sera

Serum	Dilution	Log_{10} virus neutralized by 0.015 ml serum	
		A10	A12
A10 antivirion – single inoculation into guinea pigs	1/1	4.5	1.9
	1/10	3.8	1.3
	1/100	2.5	0.9
	1/1000	1.5	0.9
A10 antipeptide – single inoculation into guinea pigs	1/4	2.7	0.5
A10 antipeptide – 3 inoculations into rabbits	1/1	4.0	0.5
A12 antivirion – single inoculation into guinea pigs	1/1	1.5	5.3
	1/10	0.5	4.1
	1/100	0.7	3.5
	1/1000	nil	2.7
A12 antipeptide – single inoculation into guinea pigs	1/1	0.5	2.5
A12 antipeptide – 3 inoculations into rabbits	1/1	2.5	>4.9

Synthetic peptides corresponding to region 141–160 were therefore synthesised and the antibodies they elicited were used in serum neutralisation tests. The results of these tests show that the antisera produced by injection of inactivated virus particles or the 141–160 peptides neutralised homologous viruses well but had only low activity against the heterologous viruses (table 1). This shows that the synthetic peptides mimic the antigenic characteristics of the intact virus. The level of neutralising activity in the sera of the animals which had received the peptide was much lower than that in the antivirus particle sera [8,9]. This may be due to other components in the virus architecture holding the major antigenic site in a conformation which the peptides do not accurately attain. Alternatively, the level of neutralising activity elicited by the coupled peptides may be a reflection of their presentation as antigens to the immune system. However, it is clear from these results that sequence 141–160 must contain sufficient information to contribute to subtype specificity.

The interpretation of these results at the molecular level relies on predictive methods. It is highly unlikely that the side chains of all the residues in the antigenic site are sufficiently exposed to participate in the antibody recognition site and those that are buried in the hydrophobic core of the protein cannot be antigenic determinants. It is possible to predict the presence or absence of specific amino acids in the protein core [14]. By combining such predictions with secondary structure predictions [15] it is possible to construct three-dimensional structural models of the antigenic site (fig.2). Both peptides are predicted to be partly helical, the helix of A10 (Ser 150–Leu 159) being slightly longer than that of A12 (Pro 153–Leu 159). The helices are disrupted by the presence of Gly 149 in A10 and Pro 153 in A12, while the deletion of Arg 145 in A10 causes a shift in the spatial arrangement of the amino acids at the N-terminus. In the intact virus the substitution of Leu for Phe at148 is unlikely to have any great influence on the folding of the polypeptide chain, since both amino acids are predicted to be in core and to have a similar distribution of dihedral angles. The same argument applies to the Ile for Leu substitution at 151, leaving the main distinction between A10 and A12 as the relative lengths of the helices and their effect on the spatial position

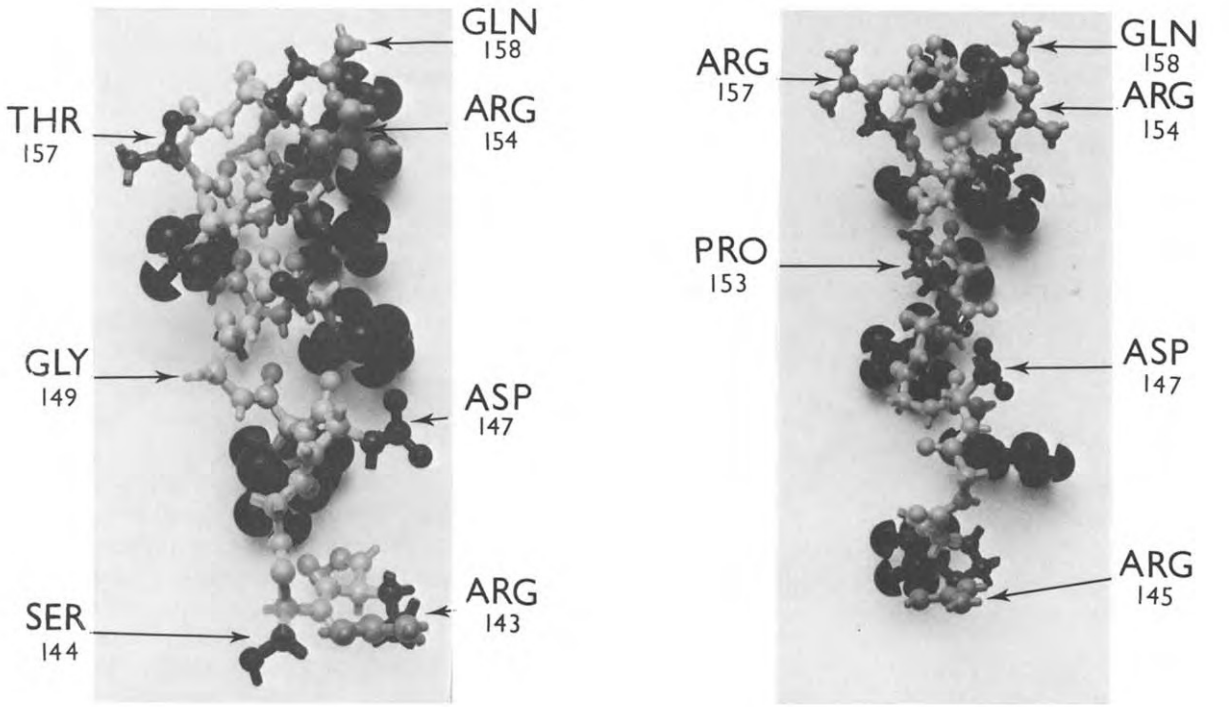

Fig.2. Sequence Arg 143–Leu 159 of serotype A10 and the corresponding sequence Val 144–Leu 159 of serotype A12 of FMDV. Residues predicted in the hydrophobic core [14] are shown with full van der Waals' radii. The orientation of groups is restricted by the need for certain of the residues to be in core (i.e., underneath) and for the overall length to be similar. The predicted helices [15] are at the C-terminal half of the sequence.

of the groups in the N-terminal region, and the substitution of Thr (A10) for Arg (A12) at position 157. Work is now in progress using chimaeric peptides from these two subtypes to determine which of these features is involved in subtype specificity.

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