Nucleotide Sequence of Gene Encoding Capsid Protein VP1 of Foot-and-Mouth Disease Virus/Type O_1 Iran

Masoudi*[1], S., Khedmati, K.[2], Parvin Ashtiani, M.[3], Khorasani, A.,[4] Shahsavandi, S.[4] and Sadri, R.[2]

1. Poultry Vaccines Research & Production Dept., Razi Vaccine & Serum Research Institute, P.O.Box 11365-1558, Tehran, Iran

2. Animal Viral Diseases Research & Diagnosis Dept., Razi Institue

3. Biotechnology Dept., Razi Institue

4. Foot-and-Mouth Disease vaccine Research & Priduction Dept., Razi Institue

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Summary

VP1 protein of foot-and-mouth disease virus (FMDV) contains the immonogenic hypervariable region of the virus. The antigenic variation in FMDV is particularly related to the difference between nucleotide and amino acid sequences of capsid protein. On the basis of this phenomenon, type diagnosis of FMDV can be done by the polymerase chain reaction (PCR). In order to specifically identify the O_1 FMDV serotype of Iran the complete coding sequence of its VP1 protein was amplified by RT-PCR, and nucleotide and amino acid sequences of the PCR product were determined. The nucleotide and deduced amino acid sequence exhibited 84% and 88% homology with the VP1 region of serotype O_1K, respectively.

Keyword: foot-and-mouth disease virus, O_1/Iran, VP1, sequence, RT-PCR

Introduction

Foot-and-mouth disease virus (FMDV), an aphtovirus, is a member of *Picornaviridae* family and causes a highly contagious and debilitation disease of cloven-hoofed animals such as cattle, sheep, goats and others. The disease is

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*Author for correspondence. E-mail: masoudi@rvsri.com

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widespread in many parts of the world and is the most economically important viral disease of live stock (Murphy *et al* 1999).

FMDV consist of a single stranded RNA molecule of positive sense and four capsid proteins (VP1-VP4) (Rueckert 1996). Like many other RNA viruses, FMDV has a high mutation rate, particularly in VP1 gene, and in the range of 10^3-10^4 substitution per nucleotide and RNA doubling (Dopazo *et al* 1988). Mutations are generated during genome replication by the viral replicase owing to lack of proof reading activity (Stram *et al* 1994). Therefor FMDV genome is highly variable; this variation leads to antigenic diversity and occurrence of seven different serotype (A, O, C, SAT1-SAT3, and Asia1) and many subtypes (Murphy *et al* 1999, Rueckert 1996). Hypervaiable region of FMDV genome that is responsible for this antigenic diversity lie in the VP1 gene segment (Acharya *et al* 1989, Bittle *et al* 1982, Dopazo *et al* 1988, Grubman *et al* 1993, Strohmaier *et al* 1982). Therefor FMDV antigenic diversity is due to nucleotide and amino acid substitution of VP1 (Beck *et al* 1987). This sequence diversity can be used in FMDV type diagnosis by RT-PCR (Callens *et al* 1997, Locher *et al* 1995, Reid *et al* 1998). In addition VP1 sequence determining would provide useful data about virus antigenicity (Stram *et al* 1994, Beck & Strohmaier 1987, Tularsiram *et al* 1997). Nucleotide sequence of the VP1 coding region of FMDVs of each of the seven serotype are now known to permit identification of the molecular basis for type and subtype classification of FMDV (Dopazo *et al* 1988). One of the main FMDV serotype that infects livestock in Iran is O_1. Here we describe the complete nucleotide and amino acid sequences of the VP1 encoding region of the FMDV type O_1/Iran genome. Morover, the relationship of the serotype to other O_1 FMDV serotypes was examined.

Materials and Methods

Virus. The seventh passage of FMDV type O_1/Iran was obtained from foot-and-mouth disease department, Razi Institute. The virus isolated from toungue epithelia

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of affected cattle in East Azarbaijan province and named O_1/95. It was propagated in BHK-21-C13 cells.

RNA. Total RNA was extracted from 500µl of FMDV infected cell suspension as previously described (Masoudi *et al* 2000).

Oligonucleotide primer. Sense and antisense primers noted previously (Masoudi *et al* 2000). cDNA synthesis was primed with a primer complementary to the 3´ end of the 1D (nucleotide sequence coding for VP1 protein) coding sequence, nucleotide 3526-3502. Sense primer was also derived from the 5´end of 1D sequence nucleotide 2886-2903. The number indicates the position of nucleotide in VP1 of O_1K (Kurze *et al* 1981).

RT-PCR and cloning. The RT-PCR condition and cloning of PCR product have been previously described (Masoudi *et al* 2000). Briefly, complete double stranded cDNA of 1D sequence was synthesized. The PCR product was purified from low melting point agarose gel as described by Sambrook *et al* (1989) and inserted by cohesive end ligation into *Eco*RI-*Nde*I site of pEt-23a+ vector. To sequence the insert the fluorescent dye deoxy-terminator system was used with the T7 promoter primer.

Serotype relationship. The relationship of O_1/Iran serotype to other O FMDV serotypes was examined on the basis of the amino acide and nucleotide sequenses of VP1. In this regard ten FMDV coding-regions were aligned and sequence base pair smilarity of them were determined.

Results

Nucleotide sequence of VP1 gene. The total length of the amplified product as per the sequense is 639bp, which correspond to the 1D segment of O_1 genome that encodes capcid protein VP1 (Figure 1). Amplification was specific because no other nonspecific product was seen in the gel. The G+C content of amplified product of

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O_1/Iran was 55.74%. The VP1 segment of O_1 serotype encodes a 639bp amino acid protein (Figure 2). The nucleotide sequence was analyzed for restriction enzyme site using computer program DNasis. There was one conserved *BstE*II site at position 436.

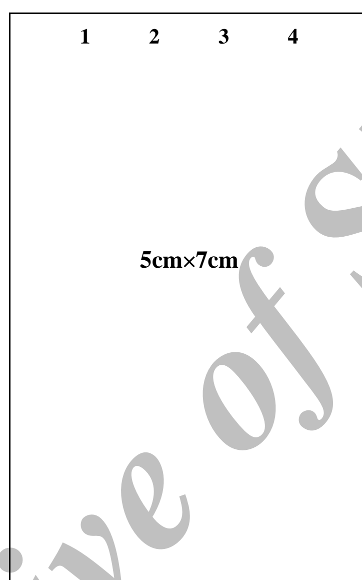

Figure 1. *The PCR product from enzymatic amplification of FMDV type O_1/Iran. Lane 1: 100bp DNA ladder, lane 2: FMDV type O_1/Iran that repeated on lanes 3 and 4*

Serotype relationship examination. The phylogenic relationship dendrogram of eleven FMDV sequences through alignment analysis of VP1 nucleotide sequences, and sequence pair distance of them were shown in figures 3 and 4. Comparison of the VP1 nucleotide sequence of O_1/Iran with O_1K (as standard virus) showed that the homology between their VP1 genes is 81.1%. The dendogram shows that the rate of nucleotide substitutions between the O_1/Iran gene and the O_1K is of 19.

Deduced amino acid sequence. The derived amino acid sequence showed that the C-terminal half of total charge is approximately +11 which confirms the earlier reports that VP1 FMDV is basic in nature. Comparsion between amino acid

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sequence of VP1 O_1/Iran with other O_1 types revale that the main alternations in amino acid sequence are located in the major antigenic domain of VP1 (Figure 5). Comparison of amino acid sequence of VP1 O_1/Iran with that of O_1K exhibited 88.7% sequence homology (Figure 6).

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     T  T  S  A  G  E  S  A  D  P  V  T  A  T  V  E  N  Y  G  G     20
1    CCACTTCTGCGGGCGAGTCGGCTGACCCTGTGACTGCCACTGTTGAGAACTATGGCGGT
     E  T  Q  V  Q  R  R  Q  H  T  D  V  S  F  I  L  D  R  F  V     40
61   GAGACACAAGTCCAAAGGCGTCAACACACAGACGTCTCGTTTATATTAGACAGATTTGTG
     K  V  T  P  K  D  Q  I  N  V  L  D  L  M  Q  T  P  A  H  T     60
121  AAAGTGACACCAAAAGACCAAATTAACGTACTGGACTTGATGCAAACCCCCGCCCACACT
     L  V  G  A  L  L  R  A  A  T  Y  Y  F  A  D  L  E  V  A  V     80
181  TTGGTGGGCGCGCTCCTCCGCGCTGCCACTTACTACTTTGCTGACCTGGAGGTTGCAGTT
     R  L  A  L  P  Y  K  H  E  G  N  L  T  W  V  P  N  G  A  P     100
241  AAACACGAGGGAAACCTCACCTGGGTCCCGAACGGGGCGCCTGAGAAGGCGTTGGATAAC
     E  K  A  L  D  N  T  T  N  P  T  A  Y  H  K  A  P  L  T  T     120
301  ACCACCAACCCAACAGCATACCACAAGGCACCGCTCACCCGGCTTGCACTGCCTTACACG
     A  P  H  R  V  L  A  T  V  Y  N  G  N  C  R  Y  G  E  G  P     140
361  GCACCACACCGTGTGTTGGCTACTGTTTACAACGGGAACTGTAGGTACGGTGGAGGCCCT
     V  A  A  V  R  G  D  L  Q  V  L  T  Q  K  A  A  R  T  L  P     160
421  GTGGCCGCTGTGAGAGGTGACCTGCAAGTGTTGACCCAGAAGGCGGCTAGAACGCTGCCT
     T  S  F  N  Y  G  A  I  K  A  T  R  V  T  E  L  L  Y  R  M     180
481  ACCTCCTTCAACTACGGTGCTATCAAGGCTACACGGGTGACTGAACTGCTTTACCGCATG
     K  R  A  E  T  Y  C  P  R  P  L  L  A  I  H  P  S  G  A  R     200
540  AAGAGGGCTGAGACATACTGCCCCCGGCCTCTTTTGGCCATTCACCCGAGCGGAGCCAGA
     H  K  Q  K  I  V  A  P  V  K  Q  T  L                          213
601  CACAAGCAAAAGATCGTGGCACCGGTGAAACAGACTTTG
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Figure 2. *The complete nucleotide sequence of the coding region of VP1 protein of FMDV type O_1/Iran*

Figure 3. *Phylogenic relationship dendogram of 11 FMDV O_1 sequenses through alignment analysis of VP1 nuclotide sequense. The number indicates the rate of nucleotide substitution*

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Figure 4. *Sequence pair distances of 11 FMDV O_1 nucleotide sequences*

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Figure 5. *Amino acide alignment of FMDV VP1. Boxes are residues that differ among the compared VP1 sequence*

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Figure 6. *Sequence pair distances of 11 FMDV O_1 amino acid sequences*

Discussion

Regular vaccination with the suitable vaccine strain is the basis of FMD control in Iran. Selection of vaccine strain needs accurate diagnostic procedure which able to detect of serotype and subtype; and also differentiate variants of a given serotype. It has been shown that nucleotide sequencing of the field viruses and comparison of

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the sequences with that of others for evaluation of the relationship degree between different viral strain may help to select a suitable vaccine strain in FMD controling program (Tulasiram *et al* 1997).

In this approach, we detect the nucleotide and amino acid sequences of the VP1 encoging region of O_1/Iran, and examine the relationship of the serotype to other FMDV O isolates. The sequencing of PCR products indicate that all products were FMDV-specific. The computer program Dnasis analyzing data on detection of restriction enzyme sites was shown that there was one one conserved *BstE*II site at position 436 which was also seen in in all O_1 subtypes (Locher *et al* 1995) and may be considered as O_1 specific site and used for detection of O_1 serotype. The G+C content of PCR product of O_1/Iran was 55.74 % which coincides with the G+C content of VP1 of FMDV serotype O1 previously reported by Tulasiram *et al* (1997). The G+C content of that serotype was found to be above 50%.

Results of our study indicate that there are 24 amino acid substitutions in VP1 gene of O_1/Iran. The amino acids between 138-160 of VP1 constitute main antigenic domain of the virus (Acharya *et al* 1989) and the amino acid between 200-213 of VP1 have been shown to responsible for the cell attachment site on FMDV for BHK cells (Tulasiram *et al* 1997). Comparison of amino acid sequences was shown that 10 of 24 alteration in amino acid sequence of O_1/Iran were located at amino acid 138-160. Highly variable region of FMDV are located at amino acid 137-156 and 210-213 of VP1. X-ray crystallographic studies showed that both sites of VP1 are exposed on the surface of the virus and are proximal to each other (Acharya *et al* 1989). Several lines of evidence suggest that 133-158 amino acid of VP1 of FMDV is highly immunodominant, perhaps due to its accessibility and structural flexibility (Acharya *et al* 1989, Logan *et al* 1993, Strohmaier *et a*l 1982). Peptides representing this VP1 segment are necessary to afford protection by synthetic vaccine formulation (Bittle *et al* 1982, Dimarchi *et al* 1986, 1988). Therefor O_1/Iran carries subtype specific amino acid in main antigenic domain of VP1 that was located on

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both sides of Arg-Gly-Asp (RGD) motif. This sequence creates the VP1 GH-loop (Acharya *et al* 1989, Logan *et al* 1993, Strohmaier *et al* 1982). The specific O_1/Iran amino acid sequence in the GH-loop is probably responsible for the antigenic response of this virus.

In conclusion, clearly, a better understanding of antigenic variation among the circulating FMDV field isolates using molecular techniques is neccesary for selecting suitable vaccine strain (s) to control of the disease. Here, we have determined the nucleotide and amino acid sequences of the VP1 of O_1/Iran genome, however, more studies depend on nuclotide sequence analysis of other citculating FMDV types are required for the production of an effective vaccine.

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