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Structural Studies on IRES 4-2 Domain of Foot-and-mouth Disease Virus

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Abstract : Foot-and-mouth disease virus (FMDV) belongs to the aphthovirus genus within the picornavirus which has a single copy of a positive sense RNA. The translation initiation process of FMDV occurs by a cap-independent mechanism directed by a highly structured element (~435 nt) termed an internal ribosome entry site (IRES). We have designed and prepared FMDV4-2 RNA (28nt) by in vitro transcription. The 2D NMR data revealed that FMDV4-2 IRES domain RNA has a flexible loop and bulge conformation. In further study, we need to make an isotope labeled RNA sample and conduct 3D NMR experiments to completely determine the 3D structure. This study may establish a new drug design strategy to treat foot-and mouth disease.
Keywords: NMR, Cap-independent mechanism, IRES, Bulge, Loop

INTRODUCTION

Foot-and-mouth disease (FMD) severely decreases livestock production and has necessitated introduces important trade restrictions on animals and livestock products. The disease is characterized by fever and blister-like sores on the tongue and lips, in the mouth, on the teats, and between the hooves. [1]

The foot and mouth disease virus (FMDV) is one of two members of the Aphthovirus genus, within the family Picornaviridae. Picornavirus translation is initiated cap independently from an internal ribosome entry site (IRES) downstream from the RNA 5' end. IRES elements contain several conserved stem loops, a core element in their 3' region [2]

and a pyrimidine tract followed by an AUG codon at their 3' border. [3] Eukaryotic initiation factor 2 (eIF2), eIF3, eIF4A, eIF4B, and the central domain of eIF4G are required for formation of 48S initiation complexes with IRES RNA and 40S ribosomal subunits.[4] Polyprymidine tract-binding protein (PTB) binds to several IRES elements and enhances translation of FMDV. [5,6] Mutations of the IRES stem-loop 4th domain reduce binding of eIF4B and translation efficiency. [7]

The objective of this study is to determine the solution structure of the 2nd stem-loop of the FMDV 4th domain (FMDV 4-2) RNA, one of the influent IRES domains of FMDV, using NMR spectroscopy. The sequences and secondary structure of the FMDV 4-2 RNA are shown in Fig. 1. FMDV 4-2 domain is related to control of initiation of translation with eIF4B. [8] This study constitutes the first case mapping by a suitable size for an NMR study.

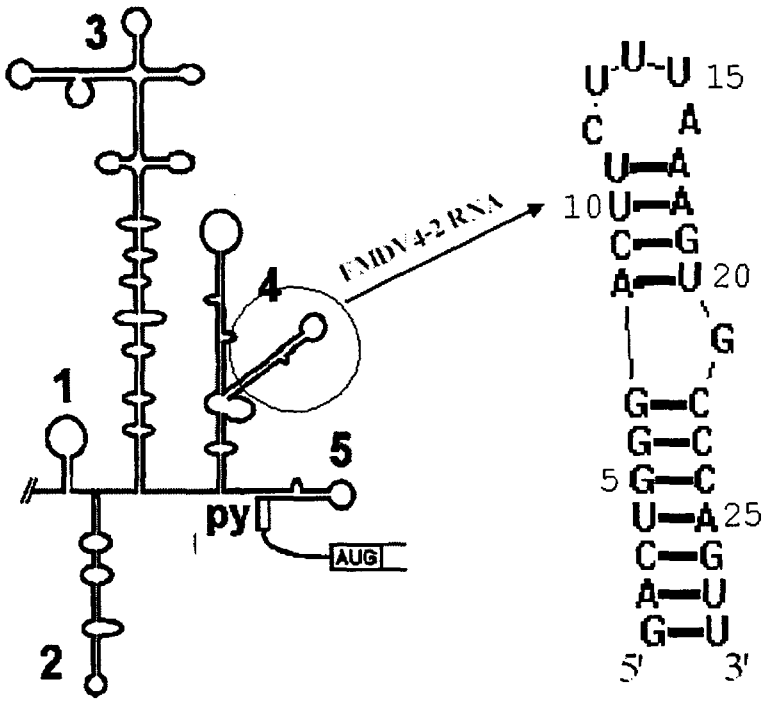

Fig. 1. Sequences and secondary structures of the FMDV 4-2 RNA

METHOD

Preparation of RNA samples

The FMDV 4-2 oligonuleotide, 5'-GACUGGGACUUCUUUAAAGUGCCCAGUU-3', was enzymatically synthesized in vitro using T7 RNA polymerase and a chemically synthesized oligodeoxyribonucleotide template. [9] The T7 RNA polymerase was obtained from a culture of over expressed Escherichia *coli* strain BL21 containing the plasmid p AR1219 carrying the gene for the enzyme. [10] The transcription product was purified using denaturing 15% polyacrylamide gel electrophoresis.

The purified RNA sample was dialyzed extensively against 10mM sodium phosphate, pH 6.6, 0.01 mM EDTA using a 1kDa cut-off Mini Dialysis Kit (Amersham Biosciences). (Spectrum). [11] The RNA sample was then recovered, brought up to approximately 1 mL with buffer, and lyophilized to dryness. For experiments involving the exchangeable protons, the sample was resuspended in 250 μL of 90% H_2O/10% D_2O. For experiments involving the non-exchangeable protons, the sample was exchanged several times with 99.9% D_2O and then resuspended in 250 μL of 99.96% D_2O. Finally, the RNA concentration was 2 mM. in 40mM sodium phosphate buffer at pH 6.6.

NMR spectroscopy

All experiments were carried out using a Bruker DRX 500 with states of the art cryoprobe spectrometers. NMR data were processed and displayed using the programs XWINNMR (Bruker) and Sparky. [12] Water suppression was achieved using a jump-return pulse with a 50μs delay between the observed pulses.[13] All experiments were measured at 298K as well as at 278K or 303K for identification of overlapped peaks. Mixing times of 50 – 400ms were used in collecting NOE spectra. Total correlation spectroscopy (TOCSY) data were also recorded in D2O with mixing times from 100-200ms using a compensated DIPSI2 sequence. A double-quantum filtered COSY (DQF-COSY) spectrum was acquired with a spectral width of 4,000 Hz, using the standard pulse sequence. [14]

A natural abundance heteronuclear ^{1}H-^{13}C multiple quantum coherence (HMQC) spectrum was acquired using a standard pulse sequence.[15,16]

RESULTS

Assignment of exchangeable protons

The imino and amino proton spectra were assigned by standard methods using one- and two-dimensional NMR spectroscopy in H_2O at 278K (Fig. 2). The FMDV4-2 RNA molecule contains seven guanosine and seven uridine residues; the 1D imino proton spectrum contains 5 sharp and 5 broad resonances out of the possible 14. The sharp resonances were assigned to the imino protons of the lower stem. These assignments were confirmed from the imino-imino cross-peaks seen between U4 and A25, G5 and C24, G6 and C23, and G7 and C22 in 2D NOESY. The upper stem and loop imino protons could not be assigned from 2D NOESY data due to the flexibility of these regions.

Assignment of non-exchangeable protons

Pyrimidine H5 and H6 resonances were identified by theirs strong cross-peaks observed in the DQF-COSY spectrum (Fig. 3). The traces of H5-H6 cross-peaks indicate the flexibility of the upper stem, particularly near the loop. Purine bases were distinguished from pyrimidine by a new pulse sequence. [17] This information provides a starting point for the assignment of non-exchangeable protons following the standard procedure based on the sequential NOE connectivity.[18] Fig. 4 shows the H8/H6/H2/-H5/H1' region of the 400-ms NOESY spectrum at 298K. The H1'-H2' region of the 50- and 100-ms NOESY and the DQF-COSY spectra was used to assign all H2' resonances. Alternate H8/H6-H2' connectivity confirmed the sequential assignments and resolved some ambiguities in the H8/H6-H1' connectivity. Adenosine H2 resonances were identified by the chemical shifts of the bound carbons in ^{1}H-^{13}C HMQC.

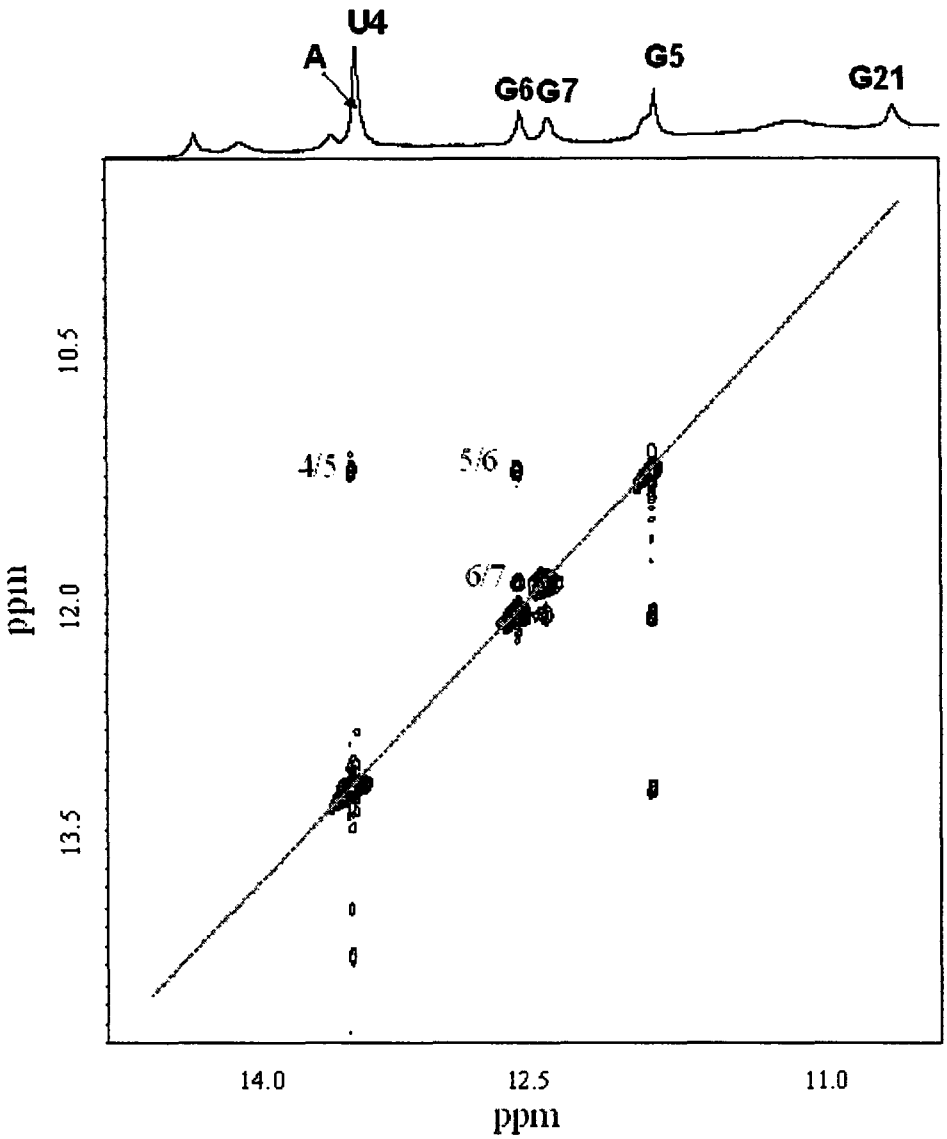

Fig. 2. A section of a 400 ms NOESY spectrum of the FMDV 4-2 RNA in H_2O showing NOEs between imino protons.

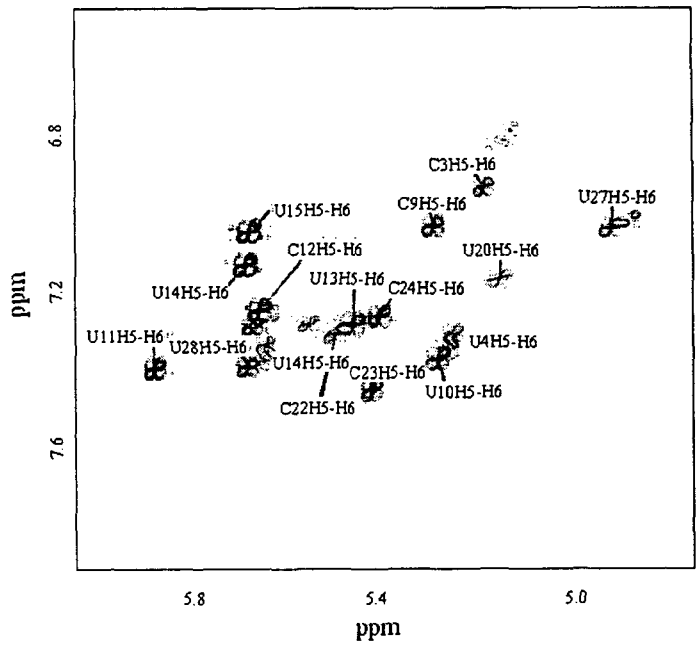

Fig. 3. A section of a DQF-COSY spectrum showing the H5'-H6' couplings.

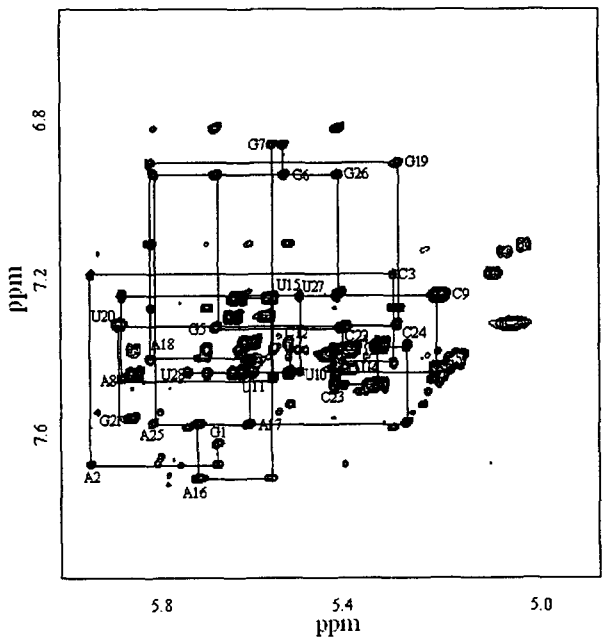

Fig. 4. Fingerprint (aromatic to anomeric) region of a 400 ms NOESY spectrum of the FMDV 4-2 RNA.

Structural Features determined by NMR

2D H_2O NOESY data shows only the sequential connectivity of imino protons in lower stem (Fig. 2). The imino peak A at 13ppm is considered to be overlapping peaks of some U and G imino protons including U4. The NOE patterns of the imino peaks contained G·C base pairs and A·U base pairs. However, the secondary structure of the upper stem following the G21 bulge was not confirmed. The broad peaks of the 1D projection shown in Fig.2 disappear at 298K. This data supports that the FMDV 4-2 RNA upper stem is very flexible. Fig. 5 displays a section of a DQF-COSY spectrum showing the H1'-H2' couplings. These cross peaks are observed on the flexible residue of the RNA structure. G1 and U28 H1'-H2' cross peaks are on terminal. From U11 to A17, the peaks are located on the loop or near the loop. From G19 to G22, cross peaks are found on or near to the G21 bulge. As such, this indicates that FMDV 4-2 RNA has a stable lower stem and flexible upper stem. The U14 and U15 shows C2'-endo sugar conformation. Other loop residues show flexible sugar conformation. From the sequential NOE connectivity, we assume the stem has A-form conformation. The stacking is continuous from the lower stem to the upper stem at the 5'-side but discontinuous at the 3'-side. The 5'-side exhibits rigid conformation whereas the 3'-side shows greater flexibility. The bulged G causes the 3'-side conformation to deviate from A-form geometry.

From the preliminary NMR experiments we suggest that the FMDV 4-2 IRES domain consists of a lower stem, a bulge, a flexible upper stem, and loop. We are currently investigating the 3D structure of the FMDV 4-2 RNA using isotope labeling of RNA. Isotope labeling of RNA will be greatly helpful in assignment and 3D structure determination of RNA.

Abbreviations

1D	**one-dimensional**
2D	**two-dimensional**
COSY	**correlation spectroscopy**
DQF	**double-quantum filtered**
HMQC	**heteronuclear multiple quantum correlation**
NMR	**nuclear magnetic resonance**
NOE	**nuclear Overhauser enhancement**
NOESY	**nuclear Overhauser enhancement spectroscopy**
ORF	**open reading frame**
TOCSY	**total correlation spectroscopy**
TPPI	**time-proportional phase incrementation**
ppm	**parts per million**

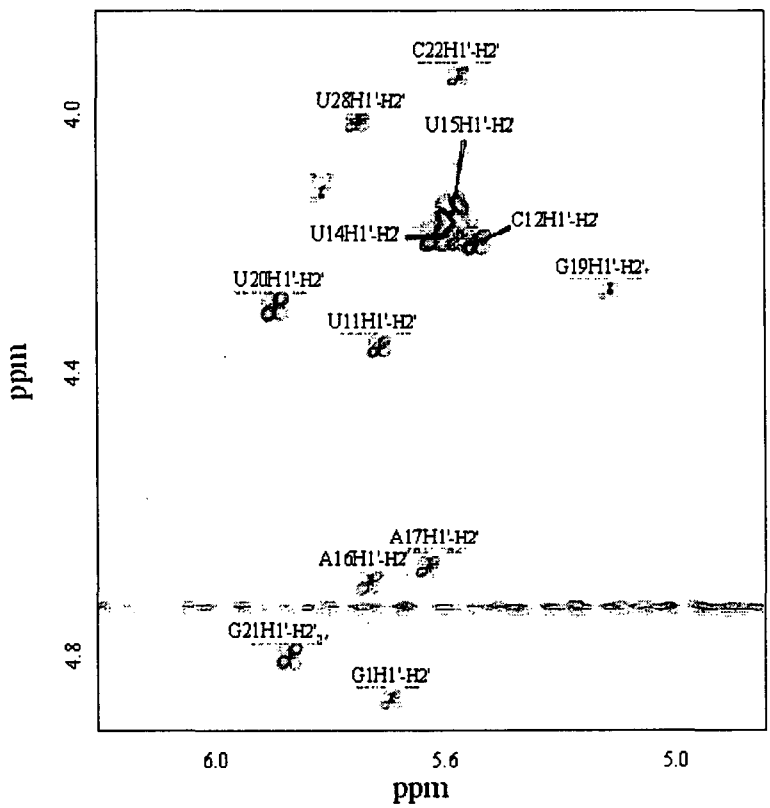

Fig. 5. A section of a DQF-COSY spectrum showing the H1'-H2' couplings

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