

Original Research Article

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Identification of Pseudoknot in the Intergenic Region between VP30 and VP24 Gene of Ebola Virus and Designing Aptamer against the Pseudoknot

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Abstract

Ebola has been identified to be one of the most ominous pathogens capable of causing pandemic fatal disease in humans. Efforts are on to control the pathogen using various different therapeutic techniques and the vaccination effort is in its early second phase according to WHO sources. However, whether the candidate vaccine can confer “herd immunity” to a particular population remains a doubt. Thus alternative therapeutic approaches should be continuously explored, so as to combat the spread of the disease. In this work we attempt to identify one of the most predominant and essential RNA structural motifs - pseudoknot in the entire *Ebola* genome. The results indicate that a conserved pseudoknot motif is present in between the intergenic regions in between VP 24 and VP 30.

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Introduction

Ebola virus, a member of Filoviridae virus family, is known as emerging and re-emerging zoonotic pathogen. It causes acute haemorrhagic fever with high case-fatality rate in humans (up to 90%) reported by Marzi et al. (2011). It was first reported in Democratic Republic of the Congo (formerly Zaire) in 1976. The virus was named after the Ebola River where it was first discovered. Five strains of Ebola virus have been identified namely- Zaire Ebola virus (ZEBOV), Sudan Ebola virus (SEBOV), Côte d’Ivoire Ebola virus (CIEBOV), Reston Ebola virus (REBOV) and the Bundibugyo Ebola virus reported by Moghdam et al. (2015). The virion is filamentous, 970nm long, 80nm in diameter and enveloped. The genome is approximately 19 kilobase negative-sense single-stranded RNA. Ebola

virus’s genomes encode seven structural proteins (namely: NP, VP35, VP40, VP30, VP24 and L) and two non-structural soluble glycoproteins (GP): Soluble GP and Small Soluble GP. Base-pairing of a single-stranded region of RNA in the loop of a hairpin to a stretch of complementary nucleotides elsewhere in the RNA chain leads to formation of a Pseudoknot studied by Cliej et al. (1985).

Pseudoknots have roles in the replication cycles of numerous animal and plant viruses, including, in humans, the Flavivirus hepatitis C virus (HCV) (Wang et al., 1995), the Coronavirus which is responsible for Severe Acute Respiratory Syndrome (SARS-CoV) reported by Thiel et al. (2003), the oncogenic retrovirus T-cell lymphotropic virus types I and II14, and certain strains of HIV reported by Brail et al. (2003).

Pseudoknots act in the regulation of initiation of protein synthesis and in template recognition by the viral replicase. In coding regions they modulate the elongation and termination steps of translation. In the 5' NCRs, these motifs have roles in the regulation of translation initiation and on the other hand in the coding region they affect translation elongation. Pseudoknots have also been identified in the catalytic RNAs of some RNA satellite viruses, where they have a role in genome replication reported by Taylor et al. (2003).

At this juncture, the present study focuses on designing aptamers against specific genomic landmarks in the Ebola genome. Development of inhibitors that target key components in pathogens often requires detailed knowledge of structure and function or screening of large compound libraries with highly sensitive reporter(s). Nucleic acid aptamers provide an alternative method for inhibitor development as well as target validation. Aptamer identification and characterization does not require detailed knowledge of the target (Binning et al., 2012; Bunka et al., 2010; Lee et al., 2006; Okonechikov et al., 2012). However, in situations where detailed structural and functional information are available, aptamer development can be tailored to enhance the impact of aptamer binding by targeting aptamer interactions to functionally important regions.

Single-stranded DNA or RNA (ssDNA or ssRNA) molecules, which can bind to pre-selected targets including proteins and peptides with high affinity and specificity, are known as Aptamers. They possess versatility in binding to diverse targets, that is, they assume a variety of shapes due to their propensity to form helices and single-stranded loops. They are used as sensors and therapeutic tools. They are also used to regulate cellular processes, as well as to guide drugs to their specific cellular targets. Their specificity and characteristics are determined by their tertiary structure, not by their primary sequence. Over the years aptamer based therapeutics have evolved as we have gained understanding regarding the methods for avoiding nuclease sensitivity, poor bioavailability, fast renal clearance and limited cellular uptake.

Materials and methods

The complete genomes of all the Ebola virus strains were curated from NCBI Genome database. Genes and Intergenic regions from each virus's genome (3'UTR, 5'UTR, GP, L, NP, VP24, VP30, VP35, VP40,

Intergenic region between VP24 and L, Intergenic region between VP30 and VP 24, Intergenic region between VP40 and GP, Intergenic region between NP and VP35) were curated separately, aligned and consensus were generated using UGENE developed by Lee et al. (2006).

Dotknot Server designed by Sperschneider and Datta (2004) was used for identifying Pseudoknot in the consensus sequences. S Oligo server designed by Ding et al. (2004) was used for designing aptamer against the Pseudoknot. The Pseudoknot and the Aptamer was docked using the PatchDock Software designed by Dunhovny et al. (2002 and 2005).

Interaction between the two macromolecules was studied using DSSR: an Integrated Software Tool for Dissecting the Spatial Structure of RNA designed by Jing and Olson (2008). The macromolecular structures were visualised using the tool RasMol developed by Roger and Milne-White (1995).

Results

A Pseudoknot was identified in the Intergenic region between VP30 and VP24 (Position 109 – 127, Estimated Free Energy = 127 Kcal/mol) (Figs.1-3; Table 1). The aptamer designed using S Oligo was docked with the Pseudoknot.

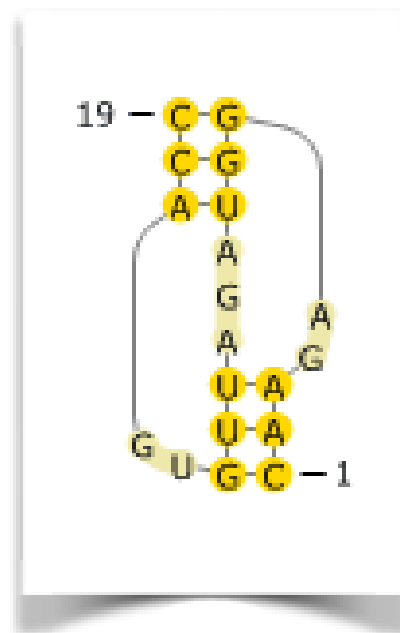


Fig. 1: Structure of Pseudoknot in Intergenic region between VP30 And VP24 visualised by using Pseudoviewer developed Han and Byun (2002 and 2003).

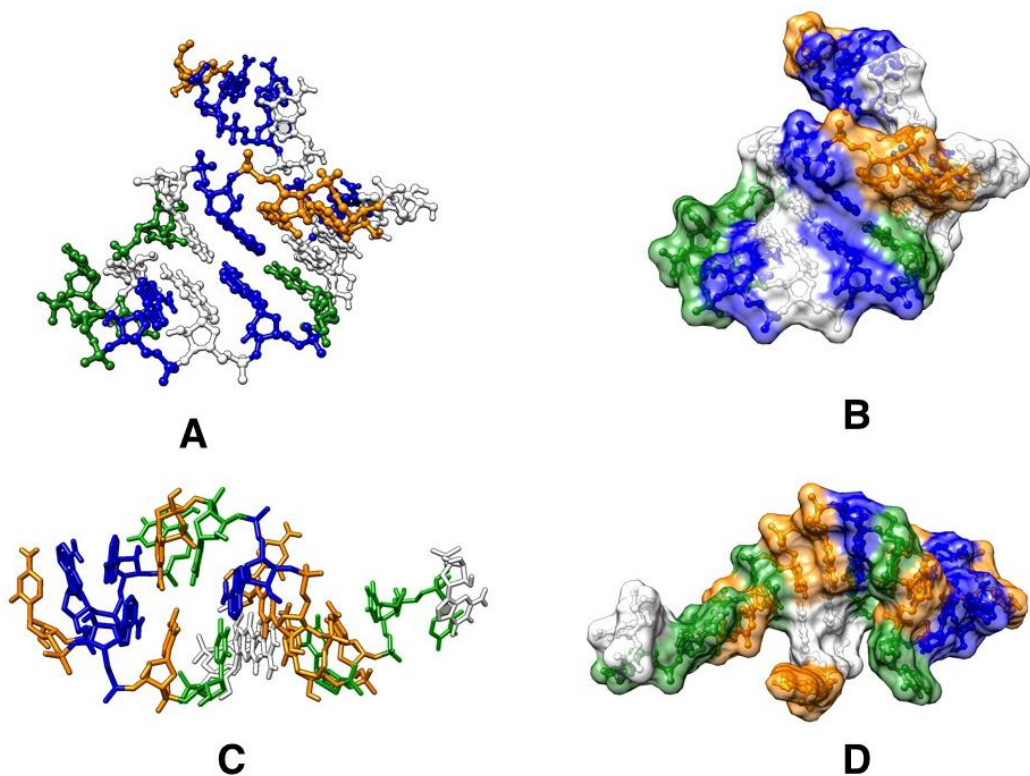


Fig. 2: Pseudoknot between VP 30 and VP 24 showing backbone and accessible surface area (A and B) and designed aptamer against the pseudoknot showing its backbone and accessible surface area (C and D). Adenine: blue, Cytosine: orange, Guanine: white and Uracil: green.

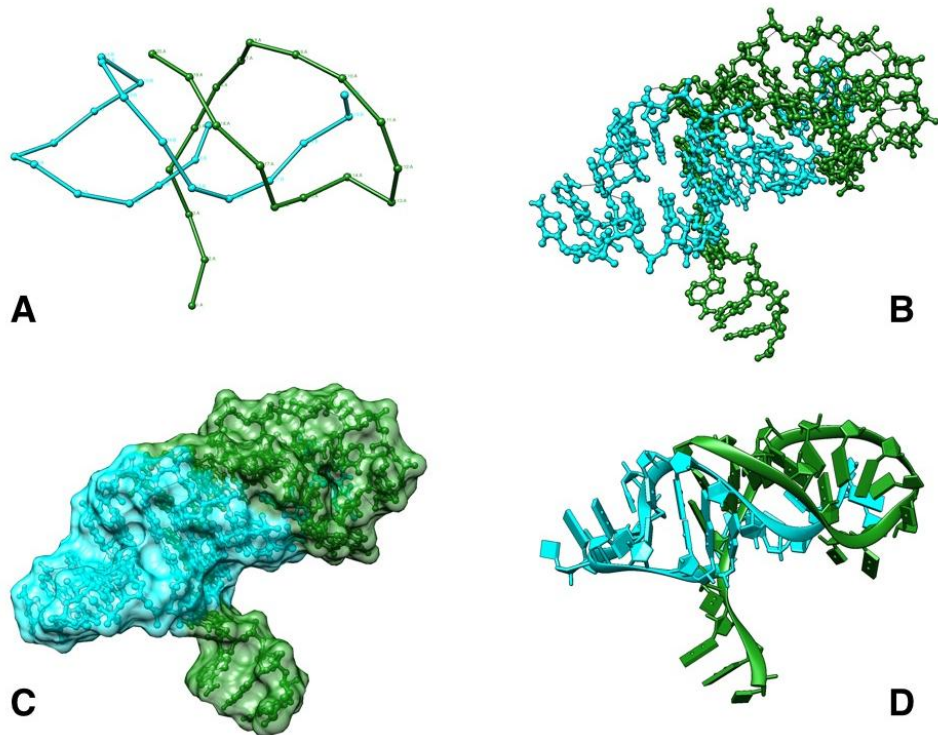


Fig. 3: Docking complex of pseudoknot (green) and aptamer (cyan). Backbone trace (A), Ball and Stick with hydrogen bonds in black (B), Ribbon showing conformation of interactions (C) and Surface Interactions (D). After analysing the docking result using DSSR, thirteen base pairings were found between Pseudoknot and Aptamer.

Table 1. List of base pairings between pseudoknot and aptamer.

Pseudoknot	Aptamer
A5	U18
G6	C15
G6, G20	U16
G7	G3, C14
A9	U12
G10	C5
G10	A6
G16	C5
A17	U4
C18	G3
C19	G2

Discussion

Over the years RNA pseudoknots have been most studied and researched structural motifs in RNA biology. It is found in almost all classes of RNA and was first identified in Turbidity Yellow Mosaic Virus, Plant viral genomes, as reported by Brierley et al. (2007). As a structure it is composed of several distinct topologies out of which the most characterized is the H-type where the bases of the hairpin loop in pair with external stem bases resulting in a second stem loop structure conformally forming the pseudoknot as indicated in Staple and Butcher (2005). Dey et al. (2011) have reported the presence of these ancient regulons in Human snRNPs. Chen et al. (2009) have reported the role of pseudoknot structures in translation initiation, elongation and frame shifting. Solinska (2014) investigated the therapeutic targeting of RNA structural motifs in RNA viral genomes and identified pseudoknots to be the most abundant RNA motifs. The identification of this pseudoknot structure in the Ebola viral genome is of significant importance as not only it sheds light on translational control and expression in Ebola but also emphasizes the regulatory function of such a common motif in the genome.

Conclusion

A Pseudoknot motif was identified and aptamer was designed against it. Significant interactions between the Aptamer and Pseudoknot was observed which indicate that the designed aptamer may serve as effective therapeutic leads for inhibiting the transcription, normal genome function as well as pathogen - host interactions. This should be clinically tested and subsequently evaluated for the same.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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