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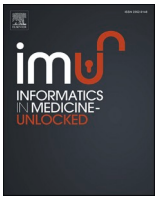

In silico design of quadruplex aptamers against the spike protein of SARS-CoV-2

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ABSTRACT

Nucleic acid aptamers are short sequences of nucleic acid ligands that bind to a specific target molecule. Aptamers are experimentally nominated using the well-designed SELEX (systematic evolution of ligands by exponential enrichment) method. Here, we designed a new method for diagnosis and blocking SARS-CoV-2 based on G-quadruplex aptamer. This aptamer was developed against the receptor-binding domain (RBD) region of the spike protein. In the current study, ten quadruplex DNA aptamers entitled AP1, AP2, AP3, AP4, AP5, AP6, AP7, AP8, AP9, and AP10 were designed *in silico* and had high HADDOCK scores. One quadruplex aptamer sequence (AP1) was selected based on the interaction with RBD of SARS-CoV-2. Results showed that AP1 aptamer could be used as an agent in the diagnosis and therapy of SARS-CoV-2, although more works are still needed.

1. Introduction

Aptamers are short single-stranded DNA or RNA oligonucleotides with complex tertiary structures capable of binding to their targets with high specificity [1]. G-quadruplex oligonucleotide aptamers are relatively more stable compared to the usual aptamers against nuclease degradation [2]. Using Antiviral aptamers is the most progressive method in the diagnosis and treatment of viruses. During the recent decade, some aptamers for SARS-CoV and MERS-CoV have been considered as potentially useful diagnostic agents and promising for detecting viruses [3,4].

Application of *in silico* methods has revolutionized the field of molecular biology by presenting valuable predictions of biological systems, and this field is growing by advent of new computational tools such as machine learning approaches [5]. Application of molecular docking for prediction of molecular interactions has helped scientists to present valuable information about the biological systems [6,7].

The SARS-CoV-2 spike protein is a homotrimeric complex, which is essential to the entry of the coronavirus into host cells, and it is one of the vital drug targets for COVID-19 [8]. The spike protein is formed into an S1 and S2 subunits [9]. The S1 domain contains the receptor-binding domain (RBD) and the *N*-terminal domain (NTD). The S2 subunit has two heptad repeat domains (HR1 and HR2) and fusion peptide [10].

One research reported some aptamers against the binding domain of SARS-COV-2 spike glycoprotein [11]. As far as we know, there is no report about developing SARS-CoV-2 quadruplex aptamer-based on spike protein. Recently, bioinformatics was used to design new aptamers and improve the binding characteristics of aptamers. Some studies have reported *in silico* approaches for modeling of aptamers against estrogen receptor alpha (ERα), the vascular endothelial growth factor (VEGF), and carcinoembryonic antigen (CEA) [12–15].

The main purpose of this work was to introduce of a new approach for the diagnosis and therapy of SARS-CoV-2. In the proposed *in silico* approach, a G-quadruplex ssDNA aptamer against receptor binding domain of SARS-CoV-2 is designed from a random pool of aptamer sequences based on the docking score, bio-conjugate free energy, and protein-ligand interaction profiler. Fig. 1 represents the flow chart of the different steps of *in silico* methods.

2. Materials and methods

2.1. Data collection

In the case of predicting potential aptamers, a G-quadruplex aptamer pool including 100 random DNA sequences with 24, 30, 31and 40 nucleotides were collected from the QGRS database. Regarding the SARS-CoV-2 spike protein, a structure with a resolution of 2.80 Å, identified by Electron Microscopy method with PDB ID: 6vxx was fetched from the protein data bank (PDB).

2.2. Structural modification of aptamers via different mutation

Different types of mutations include duplication, truncation, four-based pieces' translocation, and loop translocation were used on these aptamers to create a new ssDNA sequences library containing 10500. The structures of these quadruplex aptamers were confirmed by QGRS MAPPER [16].

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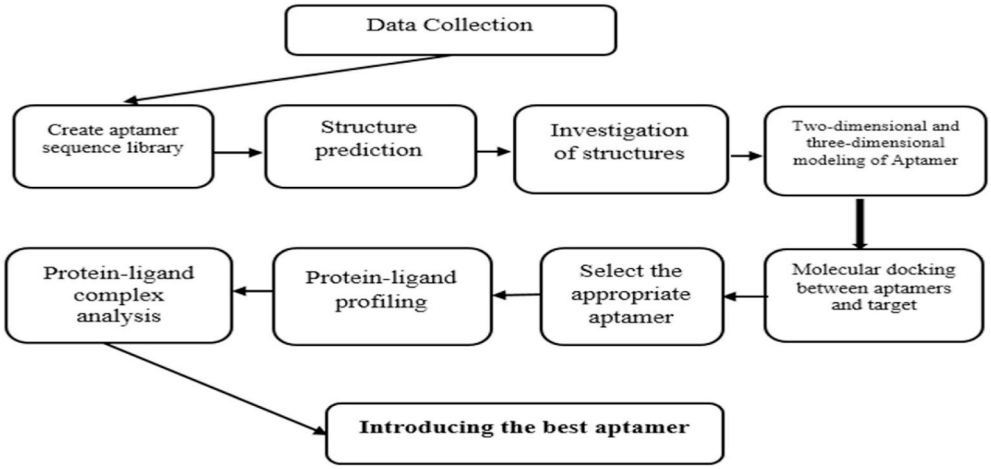

Fig. 1. A Flowchart describing the overall methods used for *in silico* analysis of the interactions between of SARS-CoV-2 spike protein and introduced aptamers to choose the most effective ligand.

Table 1
Selected sequences and predicted related parameters of the aptamers using the Mfold web serve.

Aptamer	Sequence	Length (nucleotide)	GCPercentage	Predicted free Energy ΔG (kcal/mol)	Predicted Enthalpy ΔH (kcal/mol)	Predicted Entropy ΔS (cal/k.mol)	Predicted melting point (°C)
AP1	**AAGCGGTTTTGGCCGGGGTTAAGGTTGCGG**	30	60.0	−2.67	−38.80	−116.2	60.7
AP 2	**ATCCGGGATAGGATTCTTAAGCCCTGGGCCCTGGGCCCCG**	40	65.0	−6.44	−108.9	330.3	56.4
AP3	**AAGCGTGGTTCCCCGGCCGGAAGGTTGCCA**	30	66.0	−1.64	−35.0	−107.5	52.2
AP 4	**ACAGCACGAGGGCGGGTGGGTCGG**	24	75	−1.03	−25.5	−78.8	50.0
AP 5	**AAGGGTGGTTCCCCGGCCTTAAGGTTGGCA**	30	60.0	−5.18	−60.60	−178.6	65.9
AP 6	**AAGGGTTTTTCCCCGGCCGGAAGGTTTTCCA**	31	54.0	−4.47	−65.0	−195.1	59.9
AP 7	**AAGGGTTTTTGGCCGGCCGGAAATTTTCCA**	30	50.0	−2.10	−61.70	−192.1	47.9
AP 8	**AAGCGTGGTTGGCCGGCCGGAAGGTTGCCA**	30	66.0	−4.21	−61.70	−185.3	59.7
AP 9	**AAGCGGTTTTGGCCGGCCTTAAATTTGCGG**	30	53.0	−2.76	−38.8	−116.2	60.7
AP 10	**AAGCGGTTTTGGCCGGCCTTAAGGTTGCGG**	30	60.0	−2.76	−38.8	−116.2	60.7

2.3. Predicting the structure of DNA aptamers

After that, 2-D and 3-D structures of these aptamers were predicted. By use of these structures, further studies performed about the interaction between aptamers and spike protein of SARS-CoV-2. Secondary structures of modified ssDNA aptamers were predicted using the Mfold server (version 3.1, http://unafold.rna.albany.edu) [17]. Three dimensional structure of aptamers was predicted using Rosetta server (https://rosie.rosettacommons.org/rna_denovo), according to the proposed method by Heiat et al. for predicting the structure of DNA aptamers [18,19].

2.4. Molecular interactions assay

Molecular docking is an *in silico* method which has showed to be an effective method for predicting the molecular interactions [20]. PDB files of SARS-CoV-2 spike protein (PDB ID: 6vxx) and aptamers were used as input receptor and ligand molecule in HADDOCK online web server, respectively (https://wenmr.science.uu.nl/) [21,22]. Ten aptamers with high score affinity were chosen and used for further analysis. Protein-ligand interaction profiler server (PLIP; https://projects.biotec.tu-dresden.de/plip-web/plip/index) [23] was used to visualize the interacted residues of both ligand and receptor molecules.

3. Results

3.1. Structure prediction of the potential effective aptamers against spike protein

Firstly, ten designed aptamers with 24–40 nucleotides were selected (Table 1). The HADDOCK scores of aptamer-spike protein complexes (Chains A, B and C), related Z-score, and energy parameters are shown in Table 2. Based on these results, spike protein complexes with AP2 among 40 nucleotide aptamers, AP1, AP5, and AP8 among 30 nucleotide aptamers had high percentage interactions with receptor-binding domain (RBD) and high dock scores and lowest free energy than the other complexes and were selected for further consideration.

The results (Fig. 2) showed that all modified aptamers had a hairpin structure with one or two loops followed by one or two stems. The modifications can be classified according to changes in the 3' loop, stem, and flank size. The results demonstrated that AP1 and AP5 aptamers had one loop in the 5' end. As shown in Fig. 2, aptamers AP2 had two loops at 5' and 3' ends with two stem structures, and AP8 had two loops on the top of each other in 3' end.

Table 2
Selected aptamer, dock score for all chain A, B, and C, Z-score, and related energy parameters using Haddock web server.

AP 10	6.20	−68.8	−61.2	8.3	2.3	4.2	−67.1	−107.1	98.4	−175.5	−305.9	−268.0	4.5	24.4	8.8	−2.4	−2.3	−1.8
AP 9	−28.5	−91.2	−94.9	0.2	1.0	0.8	−57.9	−86.6	−93.2	−144.2	−245.3	−234.9	12.5	19.3	19.4	−1.8	−1.7	−1.9
AP 8	−42.0	−87.4	−91.6	7.0	8.3	8.0	−64.2	−68.6	−59.3	−248.0	−441.6	−609.9	17.0	22.3	31.8	−1.5	−1.6	−1.5
AP 7	−19.3	−57.6	−59.8	0.1	8.5	8.0	−44.5	−82.0	−66.0	−432.7	−285.6	−346.4	21.1	20.9	17	−1.7	−1.7	−1.6
AP 6	−39.4	−85.8	−83.0	6.3	7.0	6.6	−70.1	−89.9	−79.0	−108.0	−273.6	−378.9	13.2	19.2	28.8	−0.9	−1.2	−1.1
AP 5	−48.4	−108.4	−102.7	0.2	0.7	1.4	−67.8	−96.0	−87.0	−346.4	−447.0	−603.6	30.8	31.1	33.8	−2.4	−2.2	−1.7
AP 4	−46.0	−76.0	−84.6	3.9	7.0	6.8	−48.2	−69.7	−75.7	−173.3	−150.6	−178.1	12.0	9.7	10.7	−1.7	−1.3	−2.0
AP3	−93.6	−94.7	−86.7	2.8	1.1	0.8	−78.3	−73.6	−81.0	−464.5	−543.3	−413.4	28.4	35.8	29.4	−2.1	−1.9	−2.1
AP 2	−23.0	−58.4	−71.7	0.4	18.1	13.0	−70.6	−68.7	−93.4	−351.0	−572.1	−509.7	22.7	28.9	29.4	−2.4	−1.7	−2.0
AP1	0.5	−61.3	−77.2	0.1	11.0	1.0	−61.0	−76.2	−83.6	−110.8	−284.9	−283.4	23.2	0.7	−2.9	−1.8	−1.9	−2.3
Aptamer	Chain A	Chain B	Chain C	Chain A	Chain B	Chain C	Chain A	Chain B	Chain C	Chain A	Chain B	Chain C	Chain A	Chain B	Chain C	Chain A	Chain B	Chain C
	Haddock score			RMSD			Van der Waals energy (kcal/mol)			Electrostatic energy(kcal/mol)			Desolvation energy(kcal/mol)			Z-Score		

3.2. Molecular interactions analysis

The interaction sites for each aptamer and spike protein of SARS-CoV-2 were determined by PLIP online web server. The results of interacted residues of four modified sequences and spike protein are illustrated in Table 3. The results demonstrated that all aptamers almost had interactions with amino acids in RBD, NTD, and HR1 domains (Fig. 3). In the figures S1 and S2, we used the method by Patel et al. [24] applying the ligplot program to show interactions between AP1(Chain B & C) and spike protein of SARS-CoV-2. The highest interaction with the RBD domain (100%) was observed in aptamer AP1 in chains B and C (Table 3). Therefore, AP1 might be a more valuable aptamer compared to others. Figs. 3-5 indicate docking results of the aptamer AP1 with the spike protein of SARS-CoV-2.

4. Discussion and conclusion

In silico methods have been used in molecular biology for various analysis such as studying different receptors and ligands. By emerging of a global epidemic, many researchers sought to make it effective prevention and treatment strategies against COVID-19 using *in silico*, *in vitro,* and *in vivo* studies [25]. Regarding this pandemics, *in silico* methods have been used in different studies for analyzing the molecular structures of SARS-CoV-2 and even introducing potential therapeutic agents [26,27].

Previous reports have indicated that *in silico* methods such as molecular docking studies could be useful for studying the interactions between oligonucleotides and protein molecules which were proved effective for therapeutic application studies [28,29]. In the current study, a new approach has been developed to design of G-quadruplex DNA aptamer against SARS-CoV-2 spike protein. G-quadruplex DNA aptamers have G-rich sequences with the ability to form four-stranded structures that are crucial for ligand binding and biocatalysis [30].

However, the design and optimization of G-quadruplex aptamers for specific enzymes and proteins are difficult to achieve. An essential strategy for discovering novel G-quadruplex aptamers is to interfere with the binding between G-quadruplex-forming sequences and the binding proteins [31,32]. However, this is the first report about designing of a quadruplex DNA aptamer for the diagnosis and treatment of SARS-CoV-2 by targeting the receptor-binding domain. This aptamer is developed against the receptor-binding domain(RBD) region of the spike protein [33].

Our results were confirmed by QGRS MAPPER. Similar studies are performed worldwide to develop drugs that inhibit varietal steps of SARS replication [3,4]. Song and co-workers by using RBD as a target for the expansion of serial DNA aptamers and a machine learning screening algorithm in the SELEX method optimized two aptamers against SARS-CoV-2 RBD [11].

Chen and co-workers have found a new way of identifying for detection of SRAS-CoV-2 N protein using DNA aptamers. The aptamers used in their study were designed based on the aptamer that had formerly been selected for SARS-CoV N protein. They bind to the SRAS-CoV-2 N protein with great affinity [34]. In the present study, the AP1 sequence has interaction with the RBD. The modified AP1 aptamer had a hairpin structure with one loop followed by a stem. Previous results also had mentioned that the stem-loop structures concerned common attentions [35]. Therefore, AP1 aptamer could be used as an agent in the diagnosis and therapy of SARS-CoV-2, but future laboratory experiments are required.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

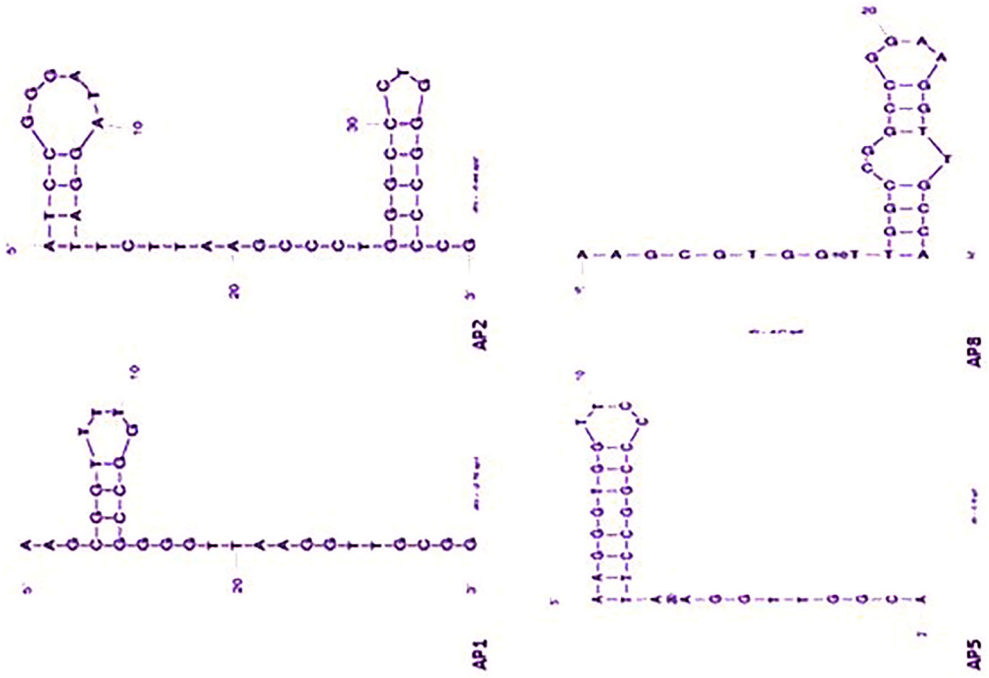

Fig. 2. Secondary structure of four selected sequences (AP1, AP2, AP5, and AP8) predicted by Mfold web server.

Table 3
HADDOCK scores, spike protein interaction domains, and interaction residues of four selected aptamer sequences.

Aptamer	Docking Score		Interaction domain	
	Chain B	Chain C	Chain B	Chain C
AP1	−61.3	−77.2	RBD (100%)	RBD (100%)
AP2	−58.4	−71.7	RBD (96.77%) other (3.33%)	RBD (93.55%), other (6.45%)
AP5	−108.4	−102.7	RBD (74.07%) NTD (25.93%)	RBD (48.38%), NTD (45.16%), other (6.46%)
AP8	−87.4	−91.6	RBD (48.5%) NTD (12.12%) HR1 (18.18%) other (21.20%)	RBD (58.34%) NTD (8.33%) HR1 (33.33%)

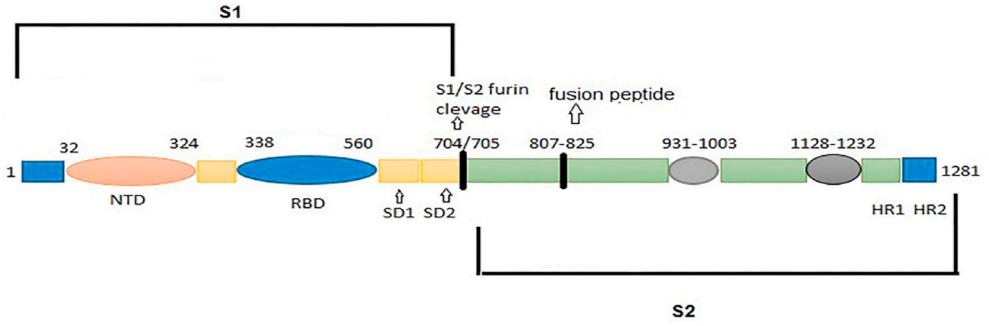

Fig. 3. Schematic presentation of SARS-CoV-2 spike protein: *N*-terminal domain (NTD), receptor-binding domain (RBD), fusion peptide (FP), heptad repeat regions 1 and 2 (HR1 and HR2).

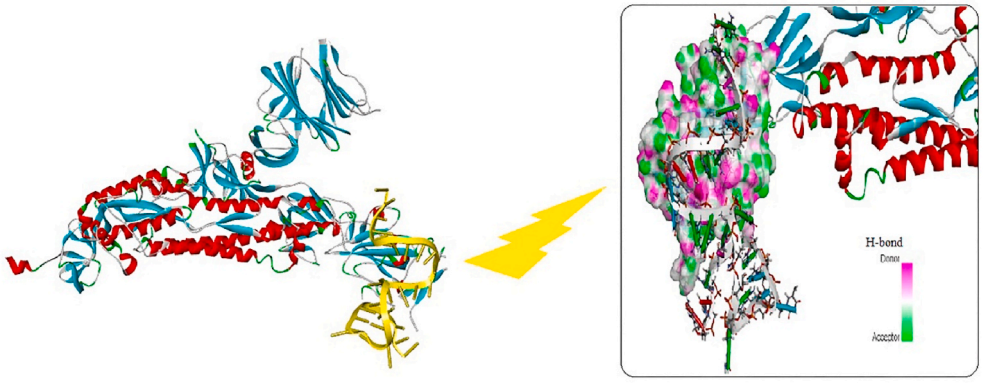

Fig. 4. A. Molecular docking complex of aptamer AP1 (yellow ribbon) with spike protein chain B and the level of its hydrogen bonds. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

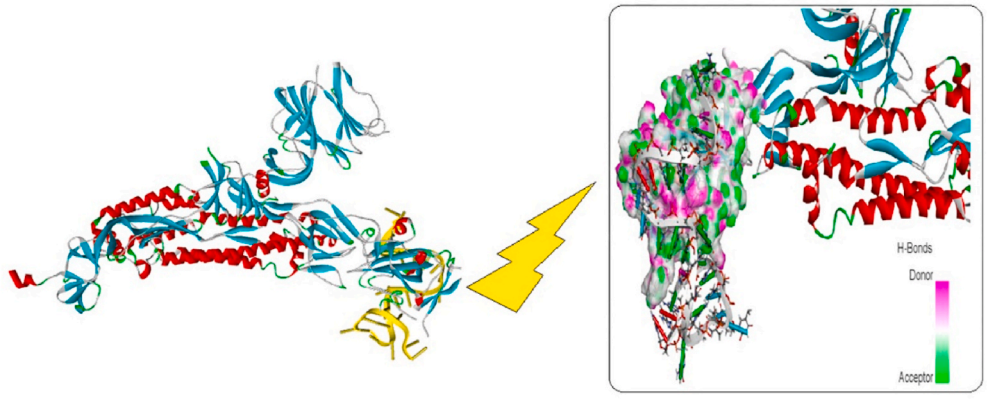

Fig. 5. Molecular docking complex of aptamer AP1 (yellow ribbon) with spike protein chain C and the level of its hydrogen bonds. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.imu.2021.100757.

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Mandana Behbahani, Hassan Mohabatkar*, Barumand Hosseini
Department of Biotechnology, Faculty of Biological Science and Technology, University of Isfahan, Hezar Jareeb St., Isfahan, 81746-73441, Iran

* Corresponding author.
E-mail addresses: h_mohabatkar@yahoo.com, h.mohabatkar@ast.ui.ac.ir (H. Mohabatkar).