Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
**In silico** design of quadruplex aptamers against the spike protein of SARS-CoV-2

**ARTICLE INFO**

**Keywords**

*In silico*

DNA Aptamer

SARS-CoV-2

Non-SELEX approach

**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].
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 (http://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://projetcs.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 3ʹ loop, stem, and flank size. The results demonstrated that AP1 and AP5 aptamers had one loop in the 5ʹ 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.
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 API (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 SARS-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 SARS-CoV-2 N protein with great affinity [34]. In the present study, the AP1 sequence has interaction with the RBD. The modified API 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, API 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.
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%) | RBD (93.55%), other (6.45%) |         |
| AP5     | 108.4    | 102.7 RBD (74.07%)| RBD (48.38%), NTD (45.16%), other (6.46%) |         |
| AP8     | 87.4     | 91.6 RBD (48.5%)  | RBD (58.34%) |         |
|         | NTD (12.12%) | NTD (6.33%)           |         |         |
|         | HR1 (18.18%) | HR1 (33.33%)         |         |         |

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

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).
Acknowledgments

The authors would like to acknowledge the University of Isfahan for the financial support of this study.

Appendix A. Supplementary data

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

References

[1] Mayer G. The chemical biology of aptamers. Angew Chem Int Ed 2009;48: 2672–89.
[2] Roxo C, Koteckiwiak W, Pasternak A. G-Quadruplex-Forming Aptamers: Characteristics, applications, and perspectives. Molecules 2019;24:3781.
[3] Shum KT, Tanner JA. Differential inhibitory activities and stabilisation of DNA aptamers against the SARS coronavirus helicase. ChemBioChem 2008;9(18): 3037–45.
[4] Seong Je Cho H-MW, Kim Ki-Sun, Oh Jong-Won, Jeong Yong-Joo. Novel system for detecting SARS coronavirus nucleocapsid protein using an ssDNA aptamer. J Biosci Bioeng 2011;112(6):535–40.
[5] Mohabatkar H, Ebrahimi S, Moradi M. Using chou’s five-steps rule to classify and predict glutathione S-transferases with different machine learning algorithms and pseudo amino acid composition. Int J Pept Res Therapeut 2021:27(1):309–16.
[6] Haghhighi O. Silico study of the structure and ligand preference of pyruvate kinases from cyanobacterium synechocystis sp. PCC 6803. Appl Biochem Biotechnol 2021: 1–21.
[7] Haghhighi O, Davaeifar S, Zahiri HS, Maleki H, Noghabi KA. Homology modeling and molecular docking studies of glutamate dehydrogenase (GHD) from cyanobacterium Synechocystis sp. PCC 6803. Int J Pept Res Therapeut 2020;26(2): 783–93.
[8] Huang Y, Yang C, Xu X-l, Xu W, Liu S-w. Structural and functional properties of SARS-CoV-2 spike protein: potential antivirus drug development for COVID-19. Acta Pharmacol Sin 2020;41(9):1141–9.
[9] Halder A, Anto A, Subramanayan V, Bhattacharyya M, Vishveshvara S, Vishveshvara S. Surveying the side-chain network approach to protein structure and dynamics: the SARS-CoV-2 spike protein as an illustrative case. Frontiers in Molecular Biosciences 2020;7:797.
[10] Wang D, Mai J, Zhou W, Yu W, Zhan Y, Wang N, et al. Immunoinformatic analysis of T- and B-cell epitopes for SARS-CoV-2 vaccine design. Vaccines 2020;8(3):355.
[11] Song Y, Song J, Wei X, Huang M, Sun M, Zhu L, et al. Discovery of aptamers targeting the receptor-binding domain of the SARS-CoV-2 spike glycoprotein. Anal Chem 2020;92(14):9895–900.
[12] Ahirwar R, Nahar S, Aggarwal S, Ramachandran S, Maiti S, Nahar P. In silico selection of an aptamer to estrogen receptor alpha using computational docking employing estrogen response elements as aptamer-alike molecules. Sci Rep 2016;6(1):21285.
[13] Chuahak Y, Stone MO. In silico selection of RNA aptamers. Nucleic Acids Res 2009; 37(12):e87–.
[14] Hu W-P, Kumar JV, Huang C-J, Chen W-Y. Computational selection of RNA aptamer against angiopoietin-2 and experimental evaluation. BioMed Research International. 2015 2015:658712.
[15] Yarizadeh K, Behbahani M, Mohabatkar H, Noorbakhsh A. Computational analysis and optimization of carcinoma-Iymphonic antigen aptamers and experimental evaluation. J Biotechnol 2019;306:1–8.
[16] Kikin O, D’Antonio L, Bagga FS. QGRS Mapper: a web-based server for predicting G-quadruplexes in nucleotide sequences. Nucleic Acids Res 2006;34(nupl): W676–82.
[17] Zuker M. Mfold web server for nucleic acid folding and hybridization prediction. Nucleic acids research 2003;31(13):3406–15.
[18] Das R, Karanicolas J, Baker D. Atomic accuracy in predicting and designing non-canonical RNA structure. Nat Methods 2010;7(4):291–4.
[19] Heiat M, Najafi A, Ranjbar R, Latifi AM, Rasaee MJ. Computational approach to analyze isolated ssDNA aptamers against angiotensin II. J Biotechnol 2016;230: 94–9.
[20] Haghhighi O, Moradi M. In silico study of the structure and ligand interactions of alcohol dehydrogenase from Cyano bacterium Synechocystis sp. PCC 6803 as a key enzyme for biofuel production. Appl Biochem Biotechnol 2020;192(4):1346–67.

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.)

Acknowledgments

The authors would like to acknowledge the University of Isfahan for the financial support of this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.imu.2021.100757.
van Zundert GCP, Rodrigues JPGLM, Trellet M, Schmitz C, Kastritis PL, Karaca E, et al. The HADDOCK2.2 web server: user-friendly integrative modeling of biomolecular complexes. J Mol Biol 2016;428(4):720–5.

Honorato RV, Koukos PI, Jiménez-García B, Tsaregorodtsev A, Verlato M, Giachetti A, et al. Structural biology in the clouds: the WeNMR-EOSC ecosystem. Frontiers in Molecular Biosciences 2021;8(708).

Salentin S, Schreiber S, Haupt VJ, Adasme MF, Schroder M. PLIP: fully automated protein–ligand interaction profiler. Nucleic Acids Res 2015;43(W1):W443–7.

Patel A, Rajendran M, Shah A, Patel H, Pakala SB, Karyala P. Virtual screening of curcumin and its analogs against the spike surface glycoprotein of SARS-CoV-2 and SARS-CoV. J Biomol Struct Dyn 2021:1–9.

Patel A, Patel A, Hemani R, Solanki R, Kamsara J, Patel G, et al. Exploring the in silico approach for assessing the potential of natural compounds as a SARS-CoV-2 main protease inhibitors. Org Commun 2021;14:58–72.

Mohabatkar H, Behbahani M, Moradi M. A concise in silico prediction report of a potential PRION-like domain in SARS-COV-2 polyprotein. J Microbiol Biotechnol Food Sci 2021. e8113–e8113, In press.

Ahmadi K, Farasat A, Rostamian M, Johari B, Madanchi H. Enfuvirtide, an HIV-1 fusion inhibitor peptide, can act as a potent SARS-CoV-2 fusion inhibitor: an in silico drug repurposing study. J Biomol Struct Dyn 2021:1–11.

Gharbavi M, Johari B, Rizmani E, Mouzazadeh N, Taromchi AH, Sharaﬁ A. NANOG decoy oligodeoxynucleotide–encapsulated niosomes nanocarriers: a promising approach to suppress the metastatic properties of U87 human glioblastoma multiforme cells. ACS Chem Neurosci 2020;11(24):4499–515.

Bigdelou Z, Johari B, Kadivar M, Rizmani E, Asadi Z, Rahmati M, et al. Investigation of specific binding of designed oligodeoxynucleotide decoys to transcription factors in HT29 cell line undergoing epithelial-mesenchymal transition (EMT). J Cell Physiol 2019;234(12):22765–74.

Bing T, Zheng W, Zhang X, Shen L, Liu X, Wang F, et al. Triplex-quadruplex structural scaffold: a new binding structure of aptamer. Sci Rep 2017;7(1):15467.

Shan C, Yan J-W, Wang Y-Q, Che T, Huang Z-L, Chen A-C, et al. Design, synthesis, and evaluation of isandinotigone derivatives to downregulate c-myc transcription via disrupting the interaction of NM23-H2 with G-quadruplex. J Med Chem 2017;60(4):1292–308.

Wang Y-Q, Huang Z-L, Chen S-B, Wang C-X, Shan C, Yin Q-K, et al. Design, synthesis, and evaluation of new selective NM23-H2 binders as c-MYC transcription inhibitors via disruption of the NM23-H2/G-quadruplex interaction. J Med Chem 2017;60(16):6924–41.

Xiu S, Liu M, Wang C, Xu W, Lan Q, Feng S, et al. Inhibition of SARS-CoV-2 (previously 2019-nCoV) infection by a highly potent pan-coronavirus fusion inhibitor targeting its spike protein that harbors a high capacity to mediate membrane fusion. Cell Res 2020;30(4):343–55.

Chen Z, Wu Q, Chen J, Ni X, Dai J. A DNA aptamer based method for detection of SARS-CoV-2 nucleocapsid protein. Virol Sin 2020;35:351–6.

Vorobyeva M, Vorobjev P, Venyaminova A. Multivalent aptamers: versatile tools for diagnostic and therapeutic applications. Molecules 2016;21(12).

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).