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Computational approach to analyze isolated ssDNA aptamers against angiotensin II

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ABSTRACT

Aptamers are oligonucleotides with highly structured molecules that can bind to their targets through specific 3-D conformation. Commonly, not all the nucleotides such as primer binding fixed region and some other sequences are vital for aptamers folding and interaction. Elimination of unnecessary regions needs trustworthy prediction tools to reduce experimental efforts and errors. Here we introduced a manipulated in-silico approach to predict the 3-D structure of aptamers and their target interactions. To design an approach for computational analysis of isolated ssDNA aptamers (FLC112, FLC125 and their truncated core region including CRC112 and CRC125), their secondary and tertiary structures were modeled by Mfold and RNA composer respectively. Output PDB files were modified from RNA to DNA in the discovery studio visualizer software. Using ZDOCK server, the aptamer-target interactions were predicted. Finally, the interaction scores were compared with the experimental results. In-silico interaction scores and the experimental outcomes were in the same descending arrangement of FLC112 > CRC125 > CRC112 > FLC125 with similar intensity. The consistent results of innovative in-silico method with experimental outputs, affirmed that the present method may be a reliable approach. Also, it showed that the exact in-silico predictions can be utilized as a credible reference to find aptameric fragments binding potency.

1. Introduction

Aptamers or chemical antibodies can act as specific ligands for plenty of targets, ranging from ions, chemical small molecules, macromolecules to even whole cells (Sun et al., 2014). They have potential properties to apply in analytical equipment, biological sensors, therapeutics, etc. (Nimjee et al., 2005). Aptamers which are developed through a procedure known as SELEX (Systematic Evolution of Ligands by Exponential enrichment) form unique three-dimensional (3-D) conformations and recognize their targets structure (Sun et al., 2014). Each aptamer ligand has unique physicochemical and binding properties. Aptamer structure is either rigid and unchangeable or flexible. In flexible cases, an aptamer can change its structure to fit with the target conformation while in rigid structures, these are targets which should be adapted to the aptamers (Hayashi et al., 2014). Deep understanding of aptamer-target interactions can be helpful for optimization and logical design of aptamers for purposeful applications. For instance, when an aptamer encounters with a small molecular target, one possibility is that the aptamer surrounds and cages it up (Hermann and Patel, 2000; Tereshko et al., 2003). This interaction model may lead to the inhibition of the target activity and is appropriate for purposeful applications (Long et al., 2008). On the other hand, aptamers which specifically recognize a region of their targets can be used as potent recognition elements in aptasensors (Hayat and Marty, 2014).To start the SELEX procedure for isolation of ssDNA aptamer, a high diversity random ssDNA library ($\sim 10^{14}$ different sequences with the same length) is required (Birch et al., 2015). A library is composed of sequences with two sides constant flanks (18–30 nt as primers) and core variable region (20–80 nt) (Lee et al., 2004). Constant flanks are recruited for PCR amplification of recovered DNA after each SELEX round. Flanks are involved in the formation of aptamer 3-D structure. The presence or absence of flanks is impressive in the overall structure and folding of the aptamer (Legiewicz et al., 2005; Shangguan et al., 2015); However, several studies have stated that certain smaller truncated

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parts of the aptamer sometimes possess an affinity higher than full-length forms (flanks + core region). Minimizing the size of the aptamer via removing the flanks and unnecessary sequences to reach the shortest region with highest affinity –specially where the aptamer has diagnostic or therapeutic applications- is very important because shortening the aptamer sequence will increase specificity and reduce the cost of production (Pan and Clawson, 2009). Finding the main binding area of aptamers, requires extensive experimental analysis and a lot of effort (Jayasena, 1999). This matter has also become a controversial issue among researchers. Using affinity mapping, mutant aptamer, chemical and enzymatic digestion of aptamers are some suggestions to find the exact target binding area (Jing and Bowser, 2011; Le et al., 2014) but trial-and-error method is time-consuming and costly. Methods such as NMR, X-ray, and Circular Dichroism (CD) can be useful for determining the structure of the aptamer and their interactions in the aptamer-target complex. Nowadays several special bioinformatic tools and computational methods are available to predict the structures and conserved motifs of nucleic acid sequences (Akitomi et al., 2011; Alam et al., 2015; Hoinka et al., 2012). Some other softwares have been introduced to predict the interaction of the aptamers with their targets and find the aptamer truncated short regions with higher affinity in a simple, rapid and cost effective way. Since RNA molecule has non-Watson-Crick base pairing and 2′-OH groups, it can form greatly more divers 3-D structures than ssDNA molecules (Sun et al., 2014), most of these tools are optimized to analyze the structure of RNA aptamers. In this study, we aimed to present a combined method that can be used to predict the 3-D structure of ssDNA aptamer and their interaction with the target. We have already isolated ssDNA aptamer against angiotensin II (Ang II) (unpublished data), which is one of the most vasoconstrictor bio-regulator and the central effector of renin angiotensin system (RAS) (Vukelic and Griendling, 2014). The present study focuses on the in-silico characterization of ssDNA aptamer and determination of the binding area within the aptamer structure and also evaluates the coordination between computational model and experimental analysis.

2. Methods

2.1. Aptamers against Ang II

Two high affinity aptamers against Ang II were isolated from a random ssDNA library (5′-CCT AAC CGA TAT CAC ACT CAC-40N-GTT GGT CGT CAT TGG AGT ATC-3′) during a strict and multistage selection procedure of SELEX, involving reiterative cycles of interaction, partitioning and amplification. At the end of the SELEX procedure, the interactions of selected aptamers with Ang II peptide were analyzed by means of surface plasmon resonance spectroscopy (SPR) system (Fig. 1).

2.2. Homology sequence analysis

Four FASTA format of the selected aptamer sequences including two full-length aptamers (each contains 82-nt) and their core regions (each contains 40-nt) were prepared and aligned together by running pairwise sequences alignment software (using Needleman-Wunsch algorithm) over the web (http://www.ebi.ac.uk/Tools/psa/emboss_needle/nucleotide.html) to find the conserved region between aptamer sequences.

2.3. 3-D structure prediction of ssDNA aptamers

Mfold web server: 1995–2015, (version 3.1, online: http://unafold.rna.albany.edu) was utilized for prediction of linear ssDNA secondary folding structure and hybridization. The folding temperature was adjusted to 25 °C. Ionic condition was provided by PBS buffer (pH 7.4) component salts containing 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 1.8 mM KH_2PO_4. Aptamer structures with the minimum energy (the lowest ΔG value) were selected. The Vienna output formats (dot-bracket notation) were used for construction of the aptamer 3-D structures. Mathews Lab RNA Structure online software (Version 5.8, online: http://rna.urmc.rochester.edu/RNAstructure.html) was utilized to visualize the 2-D structure of the aptamers. RNA Composer was used as a freely available tool over the web (http://rnacomposer.ibch.poznan.pl/Home) for entirely automated prediction of the aptamer 3-D structures. RNA Composer server acts according to the principle of translation machine and executes on the RNA FRABASE database. RNA FRABASE is a search engine associated with database of RNA 3-D structures, which uses secondary structure in the dot-bracket notation (Vienna format) as an input. Secondary structures of aptamers in the Vienna format obtained from Mfold analysis were imported into the RNA Composer server and aptamer 3-D structure outcomes were downloaded as PDB files. RNA Composer output is 3-D structure of RNA form of aptamers in which an additional hydroxyl (OH) group is present in 2′-carbon atom of ribose and also thymine is replaced by uracil. The Discovery Studio Visualizer software (windows version 2.5.5.9350) was used to modify aptamer 3-D molecular structure from RNA to ssDNA. For this purpose, aptamer PDB files were individually opened by the software, then all O2′s (oxygen attached to the 2′ carbon atom of ribose) were selected and replaced by hydrogen. In the second step to convert uracil bases into thymine, a methyl substituent was created on 5-carbon atom of all uracil bases so that the 5-hydrogens of all uracil bases (hydrogen attached to the 5-carbonatom of uracil pyrimidine ring) were replaced with carbon. Also three hydrogens were added to the newly created carbon. The modified PDB files which were changed into the ssDNA were saved. Finally, the modified aptamer structures were optimized by 32,000 steps of steepest descent energy minimization method using HyperChem molecular modeling package (Windows version 8.0.3).

2.4. Docking simulation of Aptamer/Ang II

The docking simulation was performed between each aptamer and Ang II using ZDOCK online server (http://zdock.umassmed.edu). ZDOCK as an automated tool searches all possible binding modes in the translational and rotational space between the aptamer and Ang II and evaluates each pose using an energy-based scoring function. Finally, the best pose of each docking was selected and compared with each other.

3. Results

3.1. Aptamer isolation, enzyme-linked oligonucleotide assay(ELONA) and SPR analysis

After 12 reiterative rounds of SELEX and cloning procedure, several aptamers were isolated from primary library. Among a large number of cloned sequences, two aptamers (FLC112 and FLC125) showed higher binding to Ang II peptide target comparing to others. Analysis of these two aptamers using ELONA procedure (Gong et al., 2012) revealed that they possessed high primary binding affinity to Ang II, so that FLC112 aptamer with OD = 1.92 ± 0.068, CV = 4% and FLC125 aptamer with OD = 1.74 ± 0.05, CV = 3% showed the strongest binding properties (Fig. 2). These two aptamers (FLC112 & FLC125) and their core regions (CRC112 & CRC125) were selected and synthesized for subsequent scrutinizing analysis. The calculated equilibrium dissociation constant (K_D) of the aptamers using