Research article

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3D Structural Prediction of Catechin Specific Aptamer

Nor Azlina Ahmad[1], Arinaasna Mat Tamidi[1], Razauden Mohamed Zulkifli[1,2], Huszalina Hussin[1], Muhammad Helmi Nadri[3,4]

ABSTRACT

Catechin has been reported to possess many advantageous for practical application due to its distinctive antioxidant and anti-inflammatory activities. This paper reports the *in-silico* characterization of single stranded-DNA (ssDNA) aptamers, specific for catechin. 28 primary sequences from DNA-aptamers library screened via systemic evolution of ligands by exponential enrichment (SELEX) from previous research were predicted and constructed into 3D structural conformation using several bioinformatics tools. Blind docking simulation was performed to all 28 aptamer candidates and resulted in 4 noticeable aptamers with highest binding energy arrangement from Aptamer 24, 18, 9 and 27. Influence of external factors towards catechin specific aptamers also was taken in consideration. It was predicted that these aptamers were the most potential aptamer for catechin recognition tool at laboratory scale based on the docking result. However, further *in vitro* experimental study in laboratory needs to be done as validation.

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Introduction

The catechin is one of naturally found polyphenol which is known as major component of a few medicinal [1] and various plants especially green tea and fruit-based products [2]. The most common isomer known is the (+) catechin. Due to its beneficial antioxidant characteristic, it has a wide application in health and medicine. The antioxidant and antimicrobial properties in catechin are reflected to the presence of a gallate moiety at third position of C ring which increases radical scavenging activities [3]. Besides, catechin is known due to its advantages as having hepatoprotective effect, antihypertensive, antiinflammatory, antiallergic, antithrombogenic, and hypolipidemic properties [4]. Thus, the recognition of this cellular-level compound needs to be enhanced for the sake of its advantages.

[1] Department of Biosciences, Faculty of Science, Universiti Teknologi Malaysia, 81310 Johor Bahru, Johor, Malaysia
[2] Centre of Research for Fiqh Science & Technology, Universiti Teknologi Malaysia, 81310 Skudai, Johor Bahru, Johor, Malaysia
[3] Innovation Centre in Agritechnology for Advanced Bioprocessing, Universiti Teknologi Malaysia, 84600 Pagoh, Johor, Malaysia
[4] School of Chemical & Energy Engineering, Faculty of Engineering, Universiti Teknologi Malaysia, 81310 Johor Bahru, Johor, Malaysia
*Corresponding Autor: Razauden Mohamed Zulkifli, e-mail: razauden@fbb.utm.my

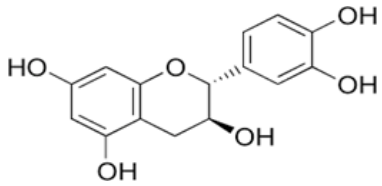


Fig 1 The 2D structure of cathechin used in this study

Therefore, a technology called aptamers is used as it can bind to their target compound through specific 3D-conformation [5]. Aptamers are short oligonucleotides with highly structured molecules which are ten times smaller than antibody, easily select, simpler and more robust than antibody-based probes. It is originated from *in vitro* experiments termed as systematic evolution of ligands by exponential enrichment (SELEX). This system is started from the libraries of random sequence and the optimization of nucleic acids to increase the binding affinity to the expected ligands. Aptamers are predominantly without structured in a solution or liquid form. Once associating with ligands, aptamers start to fold into molecular architectures and the ligand becomes nucleic acid intrinsic component [6].

Initially, the prediction of 2D structure involves the input of DNA sequences that were resulted from post-SELEX procedure or PDB online database searching with a focus to the occurrence of hairpin-like structure of single-stranded DNA (ssDNA). The process begins with prediction of ssDNA's secondary structures using Mfold web server [7]. The most thermodynamically stable structures are select from Mfold and all possible secondary structures resulted are approximately based on Watson-Crick base pairing. Construction of 3-D structure of ssDNA aptamers involved multiple software such as RNA composer [8]; Discovery Studio Visualizer and UCSF Chimera [9]. In purpose to remove deficiencies in geometric and maximize the structural parameter, the 3D RNA models then need to be refined. The ssRNA is modify into ssDNA 3D structures by detecting every uracil residue to substitute with methyl and the ribose sugar backbone replace is with deoxyribose. Final stage of the procedure involves the refinement of ssDNA 3D structures, validation of structure [10] and the predicted structure are ready to be analyzed.

Material and Methods

Structural Prediction of DNA Aptamers

The sequence of aptamers gained from previous research were converted into two-dimensional structure [11]. This process was conducted using the Mfold web server [7]. The temperature, monovalent and divalent ions concentration were adjusted to 25°C, 2 mM and 100 mM, respectively. The resulted structures were saved in portable network graphic format (png) and

Vienna file. The structures produced were validated by using Mathew Lab web server. The output from Mfold were used as an input for further analysis of 3-D structure prediction using RNA Composer. The modification method in Discovery Studio Visualizer DSV) involved several steps which include the conversion of bases (uracil into thymine) and sugars (ribose into deoxyribose). Finally, the structures were optimized at 30,000 steps of steepest descent energy minimization method and followed by 10 steps of conjugate gradient using UCSF Chimera 1.13 [9].

Docking analysis using AutoDock Vina

Digital ligands files in pdb format of catechin (compound ID: 73160) was downloaded from the PubChem database. The receptors (ssDNA aptamers) that already optimized previously was used for docking. Docking was performed in AutoDock Vina [12], which can predict interactions between small molecules and nucleic acids.

Assessment of the environmental effects of Catechin-specific aptamer

Resulted from docking process, four aptamers with the highest score of binding energy were selected as catechin-specific aptamers. Then, all of these aptamers were constructed again from their sequence by setting the parameter of temperature and other factor such as Mg^{2+} and Na^{+} using default setting of Mfold web server. The temperature, Mg^{2+} and Na^{+} default parameters were 37 ℃, 0 and 1 M respectively. The output of Mfold server of these for aptamers were then converted into 3D-DNA and were analyzed for docking analysis.

Results and Discussion

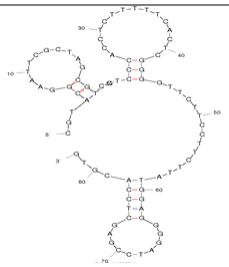
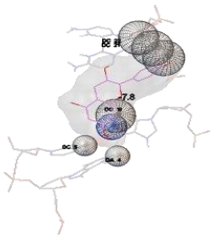
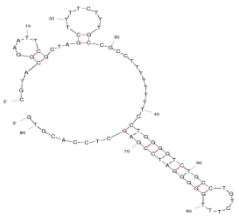
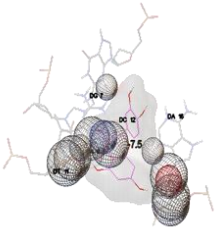
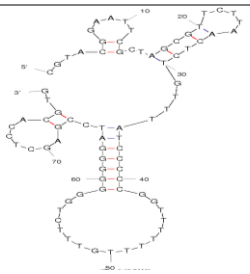
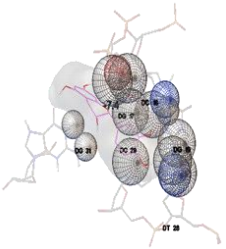
Three-Dimensional Structure and Docking of DNA Aptamers against catechin

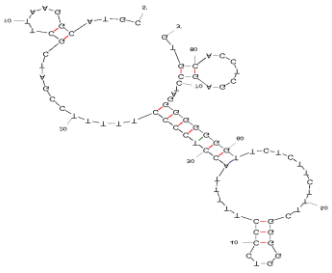
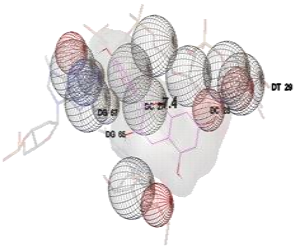
The resulted structures of aptamers were all folded to its conformation with the adjustment of the temperature at 25℃, 2 mM Mg^{2+} and 100 mM Na^{+} concentration to the most favorable condition resulting from previous *in vitro* SELEX procedure. Divalent ions such as Mg^{2+} has crucial roles in facilitate the folding of ssDNA constructed as it affects the interaction of DNA with its ligands or other protein. Furthermore, it is an essential cofactor in enzymatic system in DNA processing [13] with additional stabilizing effect. İn contrast, Na^{+} ion involves in controlling the electric field of DNA surface which affects the charge neutralization and the ion binding of the DNA structures gained [14]. The temperature were adjusted as its affect the flexibility and compactness of the DNA fold by interfering the bending of chromatin [15]. Among 28 aptamer candidates, top 4 highest binding energy with hydrogen bond location were identified and listed in Table 1.

Evaluation of the binding activity of aptamers to catechin via docking

Autodock Vina output provides the information for the H-bonds position, binding energy of the aptamer-catechin complex and nucleotides location of the ligand attachment as shown in Table 1. Each of the aptamers recorded different strength of binding affinity and binding location. These happened due to the contribution of a lots of factors such as the temperature, Mg^{2+} and Na^{+} ion concentration, Gibbs free energy and the structural conformation of the DNA aptamers. Temperature is not necessarily the only factor in aptamer performance, but it is importance in selecting the right temperature for downstream applications of aptamers. The diagnostic aptamers at room temperature should always be fixed in a range of 20–25 ˚C and the therapeutic aptamers should be static at body temperature which is 37˚C to ensure the best result for the final application of the DNA aptamers [16].

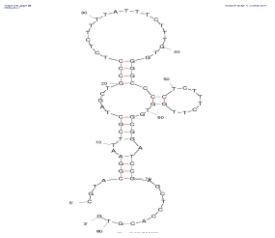
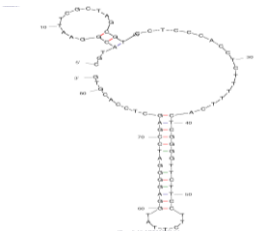
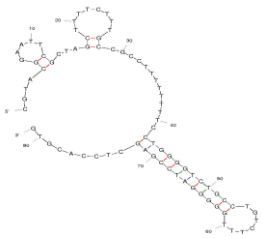
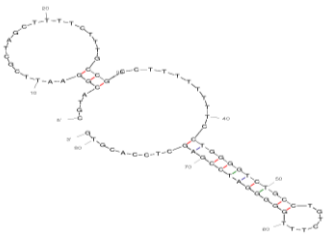
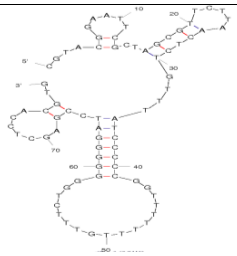
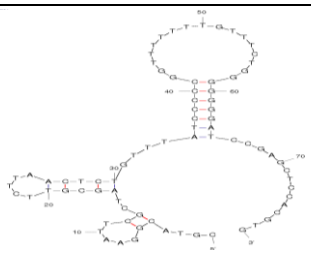
Table 1 Structural prediction and docking energy of ssDNA aptamers against catechin

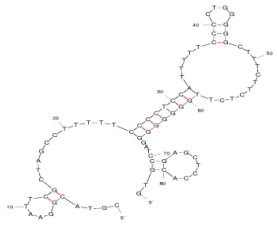
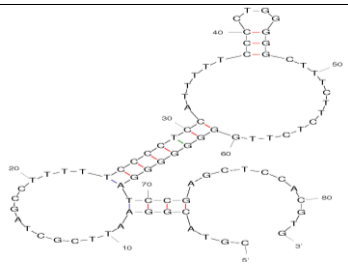
Aptamer	2D structure of possible aptamer candidates	Highest binding energy, (kcal/mol)	Site of binding	Hydrogen bond location
24		-7.8		DC5:H42
18		-7.5		Absent
9		-7.4		Absent

27		-7.4		Absent

Other than that, the concentration of Mg^{2+} and Na^{+} ions play an important part in the strength of binding of DNA aptamer. The Mg^{2+} ion is vital in facilitating the folding of aptamers and interaction of aptamers with its ligand, catechin [14]. While the Na^{+} responsible in controlling the electric charge of the DNA aptamer surface which may cause the shielding effects of negative charges of aptamer and could result in a decline of the binding affinity [17].

Table 2 Environmental assessment on catechin specific aptamer

APTAMER	**2D STRUCTURES**			
	Adjusted parameter	**Binding energy (kcal/mol)**	**Default parameter**	**Binding energy (kcal/mol)**
29		-7.8		-6.9
18		-7.5		-6.9
9		-7.4		-7.5

27		-7.4		-6.8

In addition, the structural conformation of the aptamer affect the affinity of binding and their functional contacts to catechin due to its hydrophilic polyanionic backbone and lack or exhibit weak hydrophobic interactions [18]. The binding affinity of aptamers is thermodynamically related to the Gibbs free energy (ΔG). All the structural conformation of aptamer chosen from the generated structure by Mfold web server was the one with the lowest ΔG since the more negative value is the most stable structure. Thus, when aptamer bind to ligand with a perfect fit, binding affinity could be maximally enhanced by entropic optimization. In contrast, small structural mismatches may lead to very weak binding affinity due to a negative impact on enthalpy [19]. The obtained docking results confirmed the capability of DNA aptamers possess effective molecular interaction with catechin.

Environmental assessment on Catechin specific aptamers

The assessment of the catechin specific aptamers has been carried out to observe the effect of environmental changes towards their structural conformation and activity of the DNA aptamer with its ligand. From the result observed in Table 2, the aptamers with the changed parameter structural are differ compare to the structural of the original aptamers. The binding energy of original parameter were declined as compared to changed parameters. İt might be due to the result from the folding of the structure as the aptamers experienced differentiation in the size and number of loops.

Binding affinity of hairpin DNA is mainly determined by the size of loop and the integrity of stem. Folding of DNA hairpin occurs through complicated reaction mechanism that involves both long-lived and short-lived reaction intermediates [20]. Although the hairpin loop possessed minor influence in specific site interactions on the stem, but it significantly affects the non-specific binding. In general, larger loops decreases the stability of the hairpin while smaller hairpin is more stable [21]. However, the study on this has received little attention which resulted in fewer findings in proving the effect of the loops and stem in the structural conformation of DNA aptamers towards the binding affinity.

Conclusion

The applications of DNA aptamers as biorecognition tools are expanded to its ability to detect selected flavonoids. Our study shows that the intermolecular interaction between specific DNA aptamer against selected flavonoids namely catechin, luteolin and kaempferol can provide a basic understanding on binding orientation in the complex. This was achieved by performing *in silico* approach in 3-D structure prediction and docking analysis.

Abbreviations

DNA: deoxyribose nucleic acid; RNA: ribonucleic acid; 3-D: three dimensional; PDB: Protein Data Bank; Mg^{2+}: magnesium; Na^{+}:sodium

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Conflict of 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.

References

1. Mbaveng AT, Hamm R, Kuete V. Harmful and Protective Effects of Terpenoids from African Medicinal Plants [Internet]. Toxicological Survey of African Medicinal Plants. Elsevier Inc.; 2014. 557-576 p. Available from: http://dx.doi.org/10.1016/B978-0-12-800018-2.00019-4
2. Zanwar AA, Badole SL, Shende PS, Hegde M V., Bodhankar SL. Antioxidant Role of Catechin in Health and Disease [Internet]. Vol. 1, Polyphenols in Human Health and Disease. Elsevier Inc.; 2013. 267-271 p. Available from: http://dx.doi.org/10.1016/B978-0-12-398456-2.00021-9
3. Sabaghi M, Hoseyni SZ, Tavasoli S, Mozafari MR, Katouzian I. Strategies of confining green tea catechin compounds in nano-biopolymeric matrices: A review. Colloids Surfaces B Biointerfaces. 2021;204(March).
4. Coșarcă S, Tanase C, Muntean DL. Therapeutic Aspects of Catechin and Its Derivatives – An Update. Acta Biol Marisiensis. 2019;2(1):21–9.
5. Heiat M, Ranjbar R, Fasihi-ramandi M, Mohammad A. Characterization of pharmacological properties of isolated single- stranded DNA aptamers against angiotensin II. 2016;30:238–45.
6. Hermann T, Patel DJ. Biochemistry - Adaptive recognition by nucleic acid aptamers [Review]. Science (80-). 2000;287(5454):820–5.
7. Zuker M. Mfold web server for nucleic acid folding and hybridization prediction. Nucleic Acids Res. 2003;31(13):3406–15.
8. Popenda M, Szachniuk M, Antczak M, Purzycka KJ, Lukasiak P, Bartol N, et al. Automated 3D structure composition for large RNAs. Nucleic Acids Res. 2012;40(14):1–12.
9. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, et al. UCSF Chimera - A visualization system for exploratory research and analysis. J Comput Chem. 2004;25(13):1605–12.
10. Jeddi I, Saiz L. Three-dimensional modeling of single stranded DNA hairpins for aptamer-based biosensors. Sci Rep [Internet]. 2017;7(1):1–13. Available from: http://dx.doi.org/10.1038/s41598-017-01348-5
11. Tuma Sabah J, Zulkifli RM, Shahir S, Ahmed F, Abdul Kadir MR, Zakaria Z. In vitro selection and characterization of single stranded DNA aptamers for luteolin: A possible recognition tool. Anal Biochem. 2018;549(March):72–9.
12. Oleg Trott and Arthur J Olson. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. Isr J Psychiatry Relat Sci. 2012;49(3):151–8.
13. Owczarzy R, Moreira BG, You Y, Behlke MA, Wälder JA. Predicting stability of DNA duplexes in

solutions containing magnesium and monovalent cations. Biochemistry. 2008;47(19):5336–53.
14. Tan ZJ, Chen SJ. Nucleic acid helix stability: Effects of salt concentration, cation valence and size, and chain length. Biophys J [Internet]. 2006;90(4):1175–90. Available from: http://dx.doi.org/10.1529/biophysj.105.070904
15. Driessen RPC, Sitters G, Laurens N, Moolenaar GF, Wuite GJL, Goosen N, et al. Effect of temperature on the intrinsic flexibility of DNA and its interaction with architectural proteins. Biochemistry. 2014;53(41):6430–8.
16. Sharma TK, Bruno JG, Dhiman A. ABCs of DNA aptamer and related assay development. Biotechnol Adv [Internet]. 2017;35(2):275–301. Available from: http://dx.doi.org/10.1016/j.biotechadv.2017.01.003
17. Hianik T, Ostatná V, Sonlajtnerova M, Grman I. Influence of ionic strength, pH and aptamer configuration for binding affinity to thrombin. Bioelectrochemistry. 2007;70(1):127–33.
18. Kalra P, Dhiman A, Cho WC, Bruno JG, Sharma TK. Simple Methods and Rational Design for Enhancing Aptamer Sensitivity and Specificity. Front Mol Biosci [Internet]. 2018;5(May):1–16. Available from: https://www.frontiersin.org/article/10.3389/fmolb.2018.00041/full
19. Duss O, Michel E, Yulikov M, Schubert M, Jeschke G, Allain FHT. Structural basis of the non-coding RNA RsmZ acting as a protein sponge. Nature. 2014;509(7502):588–92.
20. Jung J, Ihiy R, Scott E, Yu M, Van Orden A. Probing the complete folding trajectory of a DNA hairpin using dual beam fluorescence fluctuation spectroscopy. J Phys Chem B. 2008;112(1):127–33.
21. Lamoureux M, Patard L, Hernandez B, Couesnon T, Santini GPH, Cognet JAH, et al. Spectroscopic and structural impact of a stem base-pair change in DNA hairpins: GTTC-ACA-GAAC versus GTAC-ACA-GTAC. Spectrochim Acta - Part A Mol Biomol Spectrosc. 2006;65(1):84–94.