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ssDNA aptamers that recognize diclofenac and 2-anilinophenylacetic acid

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ABSTRACT

A series of 56 ssDNA aptamer variants that bind to diclofenac (DCF) were selected from an initial pool of 2.4×10^{14} ssDNA molecules by Flu-Mag SELEX process. Sequence analysis of these aptamer variants showed three major groups based on sequence similarity in their random N40 sequences. Out of these, four aptamers designated as D10/DA24, D22, D16, and D3 showed high affinity to DCF with K_d values 100.64, 166.34, 148.73, and 42.7 nM, respectively. Secondary structures of these aptamers showed highly distinct features with typical stem and loop structures. Specificity tests with these four aptamer variants showed that D3 aptamer had higher specificity to DCF followed by 2-anilinophenylacetic acid (2APA), a structural analog of DCF. Whereas aptamers D16 and D22 showed higher specificity to 2APA compared to DCF as target used during selection process. Further, the D10/DA24 aptamer showed high affinity but no specificity to DCF. The DCF aptamers selected can be potential candidates for drug-delivery systems, specific detection of DCF and its derivatives in pharmaceutical preparations and contaminants.

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1. Introduction

Aptamers are ssDNA/RNA oligonucleotides with high affinity and specificity for their target molecules. The concept of nucleic acid ligand was emerged from studies on viruses. In the 1980s, HIV and adenovirus researchers discovered small, structured RNA that bind to proteins with high affinity and specificity like antibody for regulating target protein.[1,2] The aptamer screening process termed as SELEX (systematic evolution of ligands by exponential enrichment) can screen large libraries of ssDNA or RNA to find aptamers which selectively bind to target by in vitro system.[3,4] Recently, many different modified methods for selection of aptamers have been developed from random pool of 10^{14}–10^{16} molecules.[5] The uses of aptamers in various fields, such as drug-delivery and biosensor applications have now been emerged. There is a considerable demand for development of aptamers for a variety of targets ranging from small organic molecules[6] to proteins[7] to even whole cells.[8]

Pharmaceutical drugs have attracted much of attention as suitable small organic molecule targets because of their potential applications. For example, identification of enantiomeric forms of racemic drugs, detection of trace amounts of drug contaminants in pharmaceutical preparations, detection of drug residues in contaminants, food, and water is of great importance in the fields of environment and human health. Currently available detection techniques rely on reference standards, such as in HPLC and GC–MS with exception to a very few drugs that can be sensitively detected using commercial detection kits.[9] Development of high affinity aptamers for such drugs can have high molecular recognition without the requirement of reference standards. The aptamers are highly specific and bind to target with strong affinity and selectivity and can potentially be used as biorecognition molecules in a biosensor.

One of the common drugs that have been used over the years is diclofenac (DCF). It is a nonsteroidal anti-inflammatory drug (NSAID) with analgesic, anti-inflammatory, and antipyretic properties. Its mechanism is to block the cyclooxgenase to inhibit prostaglandin synthesis.[10,11] DCF is also used for treatment of degenerative joint diseases and other arthritic conditions.[12,13] Although they are effective, their long-term use is limited by side effects include heart attack,[14,15] gastrointestinal lesions, headache, dizziness, skin rashes, edema, and hepatic and renal damage.[16,17] Its environmental effects are also found to be harmful. For example, about 95% of three vulture populations have distinguished during 10 years in India because of the residual DCF that was found in the kidney of all these dead vultures. This drug was ingested by feeding on carcasses of DCF treated livestock and caused death.[18–20] Diclofenac was also shown to arouse harm to freshwater fish species as rainbow trout.[21]

Although DCF belongs to the most frequently detected pharmaceutically active compounds in the water-cycle[22] and can cause critical side effects, until now, detection of DCF is mostly relied on conventional technique, such as HPLC.[23–28] Aptamer based biosensor can detect specific target molecule is simple and can be detected because of the aptamers high affinity, specificity and selectivity to DCF. Based on specific binding ability of aptamers,

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selected, the DCF aptamers developed in this study can be applied for electrochemical,[29–31] fluorescent,[32] and SPR based aptasensors.[33] To our knowledge, we here report for the first time, the selection of a series of DCF specific ssDNA aptamers by Flu-Mag SELEX method.[34] After nine selection rounds using DCF as a primary target molecule, we identified a total of 56 ssDNA sequence variants were identified. During these nine selection rounds, three intermediary counter selection rounds were introduced using beads coated with structurally related chemicals to avoid nonspecific binding and also recognize structurally related chemicals. Dissociation constant and specificity of all of these aptamers was determined by bead based binding assay using constant amount of DCF-coated magnetic beads against various concentrations of ssDNA aptamers.

2. Materials and methods

2.1. Immobilization of chemical targets on the surface activated magnetic beads

M-270 amine magnetic beads (Invitrogen Co., USA) were used for immobilization of diclofenac, 4-amino-3,5-dichlorobenzoic acid (ADA) and 2-anilinophenylacetic acid (2APA) by covalent binding. Covalent attachment of DCF carboxyl groups was performed as follows: 0.8 ml of DCF (5 mg/ml) was allowed to react with approximately 1×10^9 M-270 amine magnetic beads in 0.1 M phosphate buffer (pH 6.8) at rt by mild shaking for 2 h. After adding DCF, 0.5 ml of 10 mg/ml 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) and 15 mg/ml *N*-hydroxysulfosuccinimide (NHS) solution was added. The DCF coating process is the covalent coupling of the amino groups on the surface of the magnetic beads with the carboxyl group of DCF. To this, 13 μl of 1 M hydroxylamine was added in the reaction solution and incubated for 15 min at rt with mild shaking for quenching. The unbound DCF concentration was estimated by measuring absorbance at 276 nm (λ_{max} of DCF). The bound target concentration was calculated as the difference of added and unbound DCF concentration. The counter selection was performed by using M-270 amine magnetic beads coated with ADA and 2APA, and the negative selection was performed with naked M-270 amine beads (Fig. 1).

2.2. In vitro selection of DCF aptamers

The random oligonucleotide library was designed to enrich DCF aptamers. The random library comprised a central random region of 40 nucleotides (N40) flanked on either sides by 18 nucleotides primer binding regions for amplification and cloning. Random N40 region gave about 2.4×10^{14} diverse sequences. The sequences of random library and primers used were as follows: random ssDNA library (76-mer): 5′-ATACCAGCTTATTCAATT-N40-AGATAGTAAGTGCAATCT-3′; fluorescence labeled forward primer (P1): 5′-fluorescein-ATACCAGCTTATTCAATT-3′; and reverse primer (P2): 5′-AGATTGCACTTACTATCT-3′. All the oligonucleotides were synthesized and purified by PAGE (GenoTech Co., Korea).

For the aptamer selection process, 100 μl of DCF-coated magnetic beads (2×10^8) was washed thrice with 1 ml binding buffer (100 mM NaCl, 20 mM Tris–HCl pH 7.6, 2 mM $MgCl_2$, 5 mM KCl, 1 mM $CaCl_2$, 0.02% Tween 20) before each selection round. Initially, ssDNA pool was denatured by heating at 90 °C for 10 min, quickly cooled and incubated at 4 °C for 15 min followed by a short incubation for 7 min at room temperature (25 °C). This denaturation and renaturation of ssDNAs is a prerequisite before its application for the binding reaction in order to form stable three-dimensional structure. In the initial selection round, the DCF-coated magnetic beads were re-suspended in 200 μl binding buffer containing 10 μg (2.4×10^{14} of diversity) of the random 76-mer ssDNA library. The reaction mixture was incubated at 25 °C for 30 min with mild shaking, and the unbound oligonucleotides were removed by washing five times with same volume of binding buffer. The unbound fractions were pooled and followed by ethanol precipitation in presence of 5 μl 20% glycogen as a carrier and dissolved in 10 μl of EB buffer (10 mM Tris–HCl, pH 8.5).

Subsequently, the bound oligonucleotides were eluted separately by incubating the DCF–oligonucleotide complex five times with 200 μl elution buffer (40 mM Tris–HCl pH 8, 10 mM EDTA, 3.5 mM urea, and 0.02% Tween 20) at 65–70 °C for 7 min with mild shaking. The eluted ssDNA fractions were pooled and purified by ethanol precipitation and the ssDNA was dissolved in 10 μl of EB buffer and amplified by PCR using P1 and P2 primers. Fluorescence labeled forward primer allowed labeling of desired ssDNA fragment (aptamer candidate) in the duplex DNA product during PCR amplification. The fluorescently labeled ssDNA was separated from dsDNA product by resolving it on 10% polyacrylamide gel containing 6 M urea and 20% formamide in TBE buffer by denaturing PAGE.[34] The fluorescent ssDNA band separated from the dsDNA on denaturing PAGE was confirmed by visualizing under the UV-transilluminator before and after staining with ethidium bromide solution. The desired ssDNA fragments (fluorescent band) was cut out from the gel and crushed in 1 ml crush and soak solution (500 mM NH_4OAc, 0.1% SDS, 0.1 mM EDTA). The ssDNA fragments were then eluted from the gel after incubation at 37 °C for 12 h with mild shaking and subjected to ethanol precipitation and dissolved in 10 μl of EB buffer. The concentration of the unbound and bound ssDNA was measured by using a Nanodrop Spectrophotometer (ND-1000, Nanodrop Technologies, Inc., USA). The bound ssDNA concentration was calculated as the difference of the bound and unbound concentrations.

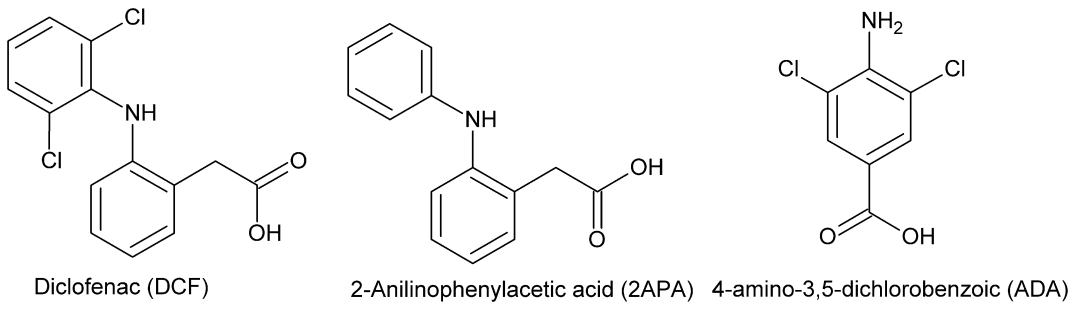

Figure 1. Chemical structures of target and counter target molecules used during SELEX process: diclofenac (DCF) was used as a primary target for selection of ssDNA aptamers. 2-Anilinophenylacetic acid was used as a counter target which is a structural analog of DCF and both were coated on amine activated magnetic beads. A structurally distinct chemical 4-amino-3,5-dichlorobenzoic acid was used as a counter target to avoid nonspecific enrichment of aptamers which was coated on carboxyl or amine activated magnetic beads.

The PAGE separated ssDNA pool from the previous round was used as the starting library for the subsequent round for further enrichment of DCF aptamer and the whole procedure was repeated until the last selection round. To avoid enrichment of nonspecific ssDNA during selection process, counter selections were performed as follows: (a) negative selection with naked M270 amine beads after second round to remove nonspecific ssDNA followed by counter selections with (b) M270 amine beads coated with ADA (also coated on carboxy-activated beads alternatively used during SELEX) and (c) 2APA after the fourth round. A total of nine selection rounds were performed. In order to increase the number of DCF binding sites, the DCF-coated M270 amine beads were increased two and three times during eighth and ninth selection rounds by using 200 μl (4×10^8) and 300 μl (6×10^8) of DCF-coated magnetic beads, respectively.

2.3. Cloning and analysis of selected library

The ssDNA pool after the last selection round was amplified by PCR using unlabeled forward primer (P3): 5′-ATACCAGCTTATTCAATT-3′ and reverse primer (P2) for cloning process. The cloning was performed using TOPO TA Cloning Kit (Invitrogen, USA) using unlabeled dsDNA product of eighth and ninth selection rounds. Plasmid DNAs carrying potential DCF aptamer candidates were purified and the aptamer insert DNAs were sequenced (Genotech Inc., Korea). The sequence similarities within random N40 regions of the aptamers were analyzed by CLUSTAL W program using a web based Internet tool (http://align.genome.jp/) and sequences were aligned using GENEDOC software, which allowed identifying conserved and consensus regions.[35] The Neighbor-Joining trees were drawn using similarity indices by MEGA 2.1 program.[36] The secondary structures of the ssDNA aptamers was analyzed by using an Internet-tool m-fold (http://frontend.bioinfo.rpi.edu/applications/mfold/cgi-bin/dna-form1.cgi) which is based on free energy minimization algorithm according to Zuker.[37]

2.4. Characterization of cloned ssDNA aptamers

The selected ssDNA aptamer candidates were tested for their affinity to bind DCF. Binding assays was performed by the method similar to SELEX method. For binding studies, a constant amount of DCF-coated magnetic beads were incubated with various concentrations of ssDNA (0–2 μM) for 30 min at 25 °C in binding buffer. The unbound ssDNA was separated by washing several times with binding buffer. Elution of bound ssDNA was carried out by incubating the DCF-coated bead–ssDNA complex in elution buffer for 7 min at 70–75 °C. The bound and unbound ssDNA concentrations were subjected to ethanol precipitation separately and dissolved each in 10 μl of EB buffer and measured spectrophotometrically using Nanodrop (ND-1000, Nanodrop Technologies, Inc.). The dissociation constants were calculated by plotting the nanograms ssDNA bound to DCF versus the initial ssDNA concentration. Alternatively, the reaction mixture was filtered through YM10 Microcon filter columns (Amicon) and centrifuged for 8 min at 12,000*g*, allowing 100 μl of solution to flow through the membrane similar to equilibrium dialysis,[38] the solution that remained above the molecular weight cutoff membrane contains free DCF, free ssDNA, and ssDNA-bound DCF, and the filtrate contained only DCF, an equivalent concentration of the free DCF in the initial solution. An 80-μl filtrate sample was used to determine the concentration of DCF by UV/Vis spectrophotometer at λ_{276}. The data points were fitted by the nonlinear regression analysis with the help of a following equation using SIGMAPLOT 8.0 software

$$y = \text{Bmax} \times \text{free ssDNA}/K_d + \text{free ssDNA} \quad (1)$$

where y is the degree of saturation, Bmax is the number of maximum binding sites, K_d is the dissociation constant.[39]

For specificity tests, the aptamers were incubated with the (a) naked M270 amine beads (negative selection) and counter selection with beads coated with (b) 2APA and (c) ADA and compared the specificity with that of DCF-coated beads. For this, 500 nM of ssDNA was incubated with constant amount beads coated with DCF, naked M270 amine beads, 2APA and ADA. The bound ssDNA fraction was recovered by elution using elution buffer followed by purification by ethanol precipitation and measured the bound ssDNA concentration. The specificity of ssDNA aptamers to the above targets was calculated as amount of ssDNA bound from the initial pool. All of the above experiments were performed in at least triplicates for error analysis.

3. Results and discussion

ssDNA aptamers that specifically bind DCF were selected from random library of 2.4×10^{14} ssDNA molecules by Flu-Mag SELEX process using magnetic beads coated with DCF. During each selection round, the ssDNA bound to DCF were eluted by salt and heat denaturation allowing recovery of strongly bound DCF aptamers. The amount of DCF bound ssDNA on the magnetic beads was enriched as the selection rounds progressed. A total of nine selection rounds were performed to obtain DCF specific ssDNA pool and three counter selection steps were introduced using (a) negative selection using naked M270 amine beads, and counter selection with beads coated with (b) 2APA, a DCF analog, and (c) ADA, a structurally distinct chemical with an aromatic ring to avoid nonspecific ssDNA binding during SELEX process (Fig. 1). The recovery of ssDNA from the initial pool added was consistent (~40–50%) from fourth to sixth SELEX round which did not increase probably because of the saturation of binding sites on the DFC-coated magnetic beads. The recovery rate after seventh round was only about 58% of the added initial DNA pool. Therefore, the initial number of DCF-coated magnetic beads was increased two and three times in eighth and ninth SELEX rounds, respectively. As a result, the recovery of ssDNA was enhanced to about 90% of the initial pool added. This result indicated that the increase in the number of binding sites increased the recovery rate of ssDNA aptamers indicating that the aptamers are strongly binding to the target (Fig. 2).

A large number of potential ssDNA aptamers specific for DCF were obtained (56 variant sequences) after cloning of the ssDNA

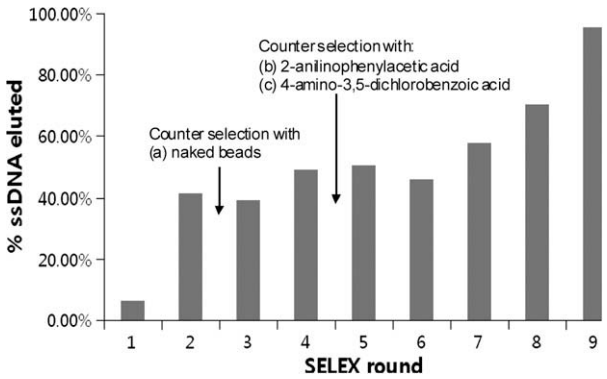

Figure 2. Selection of ssDNA aptamers that bind DCF coated on magnetic beads: elution profile of DCF bound ssDNA aptamers eluted from initial pool added after each SELEX round. For initial seven selection rounds, 2×10^8 beads coated DCF were used and the number of beads were raised to 4×10^8 and 6×10^8 beads in eighth and ninth rounds, respectively. Counter selection steps were introduced using (a) naked beads after two SELEX rounds, (b) and (c) beads coated with 2-anilinophenylacetic acid and 4-amino-3,5-dichlorobenzoic acid, respectively, after four rounds.

fractions obtained from eighth and ninth SELEX rounds. This large pool of aptamer candidates enabled selection of high affinity aptamers that are specific to DCF and/or its structural analog (2APA). Although, use of increased number of DCF-coated beads during eighth and ninth SELEX rounds enhanced the% recovery, this can also expand the possibility of low affinity ssDNA pool. In a previous study, it was shown that the less number of targets near the end of SELEX process enhanced the stringency of SELEX by strict competi-

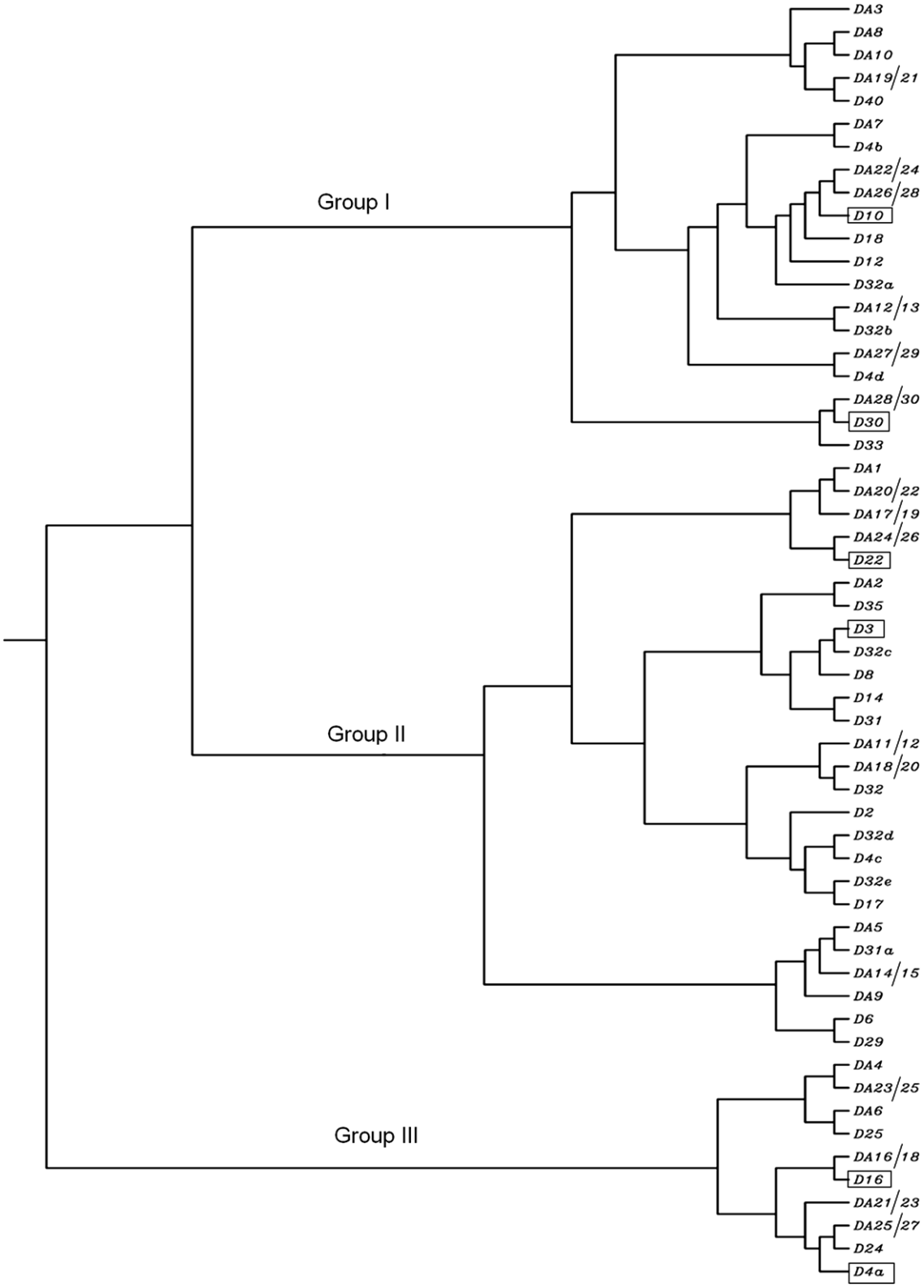

Figure 3. Dendrogram shows sequence similarity of random N40 nucleotide regions from total 56 candidate aptamer variants. Three closely related groups can clearly be distinguished from the dendrogram. Out of these three groups, six aptamer candidates were randomly picked that also covered at least one representative from three groups for further characterization.

tion.[40] However, in this study, the unbound ssDNA were eluted after treatment by mild denaturation that also ensured no enrichment of weakly bound or nonspecific ssDNAs after eighth and ninth rounds.

Two pools of ssDNA were cloned separately from eighth and ninth SELEX rounds and a total of 56 potential clones were selected and subjected to sequence analysis. The random N40 sequence region of all aptamer candidates were analyzed by CLUSTAL W for sequence similarity and m-fold analysis for prediction of their secondary structures. A dendrogram was obtained from the N40 sequences of 56 aptamer variants that showed three main groups (Groups I–III) based on sequence similarity indices. Each of these groups shared considerable number of conserved and consensus

Table 1
Sequences of random N40 regions in 76-mer aptamers (19–58th position) flanked on either sides by 18 nucleotide constant primer binding region (5′-ATACCAGCTTATTCAATT-N40-AGATAGTAAGTGCAATCT-3′)

[a] Aptamers used for characterization in this study are shown in boxes.
[b] The quantified consensus and conserved sequence regions are highlighted in dark and gray. Gaps are indicated by a hyphen (-) and those found in between the sequences were auto aligned by GENEDOC software.

sequences (Fig. 3 and Table 1). The secondary structures of tested aptamers were analyzed (Table 1; Fig. 4). Aptamers in each group category showed similar sequences and therefore share striking similarities in their secondary structures. These three groups derived from similarity indices were further analyzed to identify conserved and consensus sequences within N40 regions of the aptamers. It was found that highest N40 similarity was seen with aptamers DA22/24, DA26/28, D10, D18, and D12 followed by D32a aptamers found in Group I. These aptamers showed striking similarity in that both DA22/24 and D10 shared 100% of their N40 regions followed by DA26/28 with only an 'A' in place of G-residue at 38th position (5′–3′). While the D18, D12, and D32a had 88%, 68%, and 62% similarity, respectively. Further analysis of the sequences of these aptamers allowed elucidating the consensus and conserved sequences that are predicted to be the binding sites, which are unique to each group and found on similar or on different locations within 19–58th position of N40. The quantified similarities of conserved and consensus sequences within each group are highlighted (dark and gray) in Table 1 (see also Supplementary data). Based on the dendrogram tree obtained from 56 aptamer sequence variants and similarity classification, few potential aptamer candidates such as D4a, D10/DA24, D16, D22, D30, and D3 were arbitrarily selected that also represent at least one in each group among the variants for further characterization.

Dissociation constant (K_d value) was determined using various concentrations (0–1 μM) of selected six potential aptamer candidates (D4a, D10/DA24, D16, D22, D30, and D3) against constant number of DCF-coated beads (2×10^7). Aptamers DA24 and D10 found in eighth and ninth SELEX rounds, respectively, shared 100% similar sequences therefore designated here as D10/DA24. The DCF bound ssDNA was eluted and determined the bound ssDNA concentration. ssDNA concentration bound to DCF was plotted against the initial ssDNA pool added to determine K_d values with the help of nonlinear regression analysis (Fig. 5). The dissociation constant of D10/DA24, D22, D16, and D3 was determined to be 100.64 ± 40.5, 166.34 ± 57.9, 148.73 ± 15.5, and 42.7±15.9 nM, respectively. These selected aptamers were diverse in their N40 and thus had distinct structural features and binding affinities (Figs. 4 and 5). The amount of ssDNA bound to D22 was relatively high compared to the other aptamers (Fig. 5). This can be related to the influence of unique stem and loop structures and thus the different binding modes (Fig. 4). It was found that D30 aptamer though shared 62% similarity with D10 in N40 region but showed low affinity to DCF. The D4a aptamer showed weak binding to DCF. Two different methods for determining binding constants was employed to confirm the dissociation constants, such as binding of aptamers to (a) immobilized DCF on the beads and (b) freely suspended DCF methods. The bead based method showed a slightly higher dissociation constant than the free target system probably

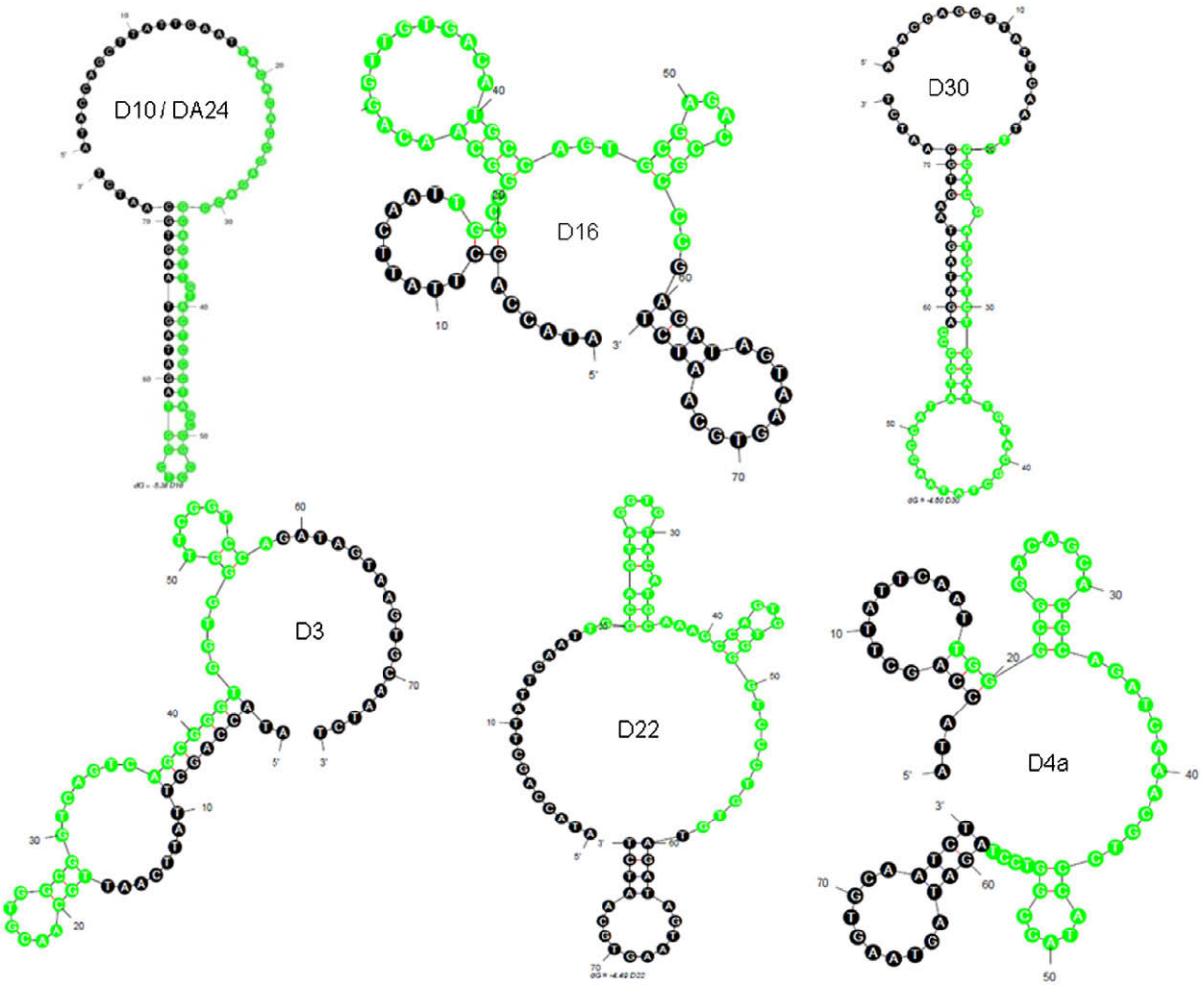

Figure 4. Secondary structures of tested diclofenac aptamers designated as D10/DA24, D16, D22, D3, D30, D4a as predicted by m-fold program. Each aptamer have 40 nt random region (N40) in the middle of the sequence (green) with 18 nt primer binding region on the flanking sides (dark).

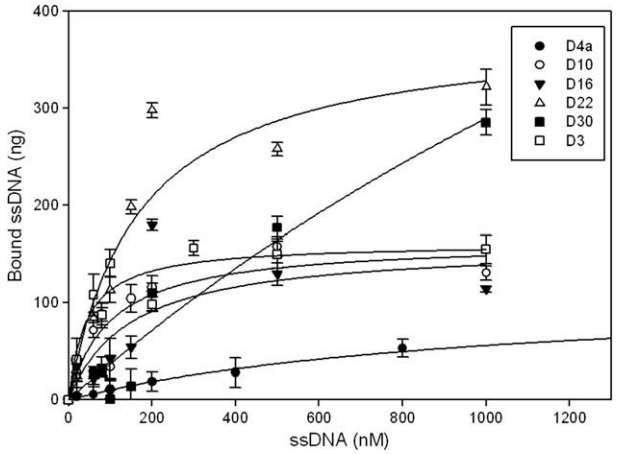

Figure 5. Bead based binding assays for determination of dissociation constants. A series of ssDNA aptamer concentrations (0–1 μM) were incubated with constant number (2×10^7) of DCF-coated beads. Six aptamers (D10/DA24, D16, D22, D3, D30, and D4a) were tested for their binding affinity with DCF. After incubation of ssDNA with DCF-coated beads, they were then washed several times and eluted the bound ssDNA using elution buffer followed by heat treatment. The data points of eluted ssDNA amounts were plotted against the concentration of the initial ssDNA pool added and K_d was determined by nonlinear regression analysis.

because the freely suspended target provide free access to binding with aptamer than the immobilized targets. However, we have not considered the results of freely suspended target because of the loss of unbound DCF during the filtration.

Specificity of the selected aptamers was tested by using naked beads and beads coated with structurally distinct and related counter targets, such as, 2APA and ADA (Fig 6). It was found that D10/DA24, D16, D22, and D3 showed distinct specificity features against the tested target molecules when compared to their affinities with DCF as the target. Although DCF was the primary target used for selection of aptamers, D22 and D16 showed higher specificity to 2APA (a structural analog of DCF lacking two chlorine atoms) followed by DCF according to their order of specificity. This result indicates that D22 and D16 aptamers probably bind on the surface of the aromatic ring of phenylacetic acid moiety of DCF molecule leaving free the aromatic ring with hydrophilic chlorine

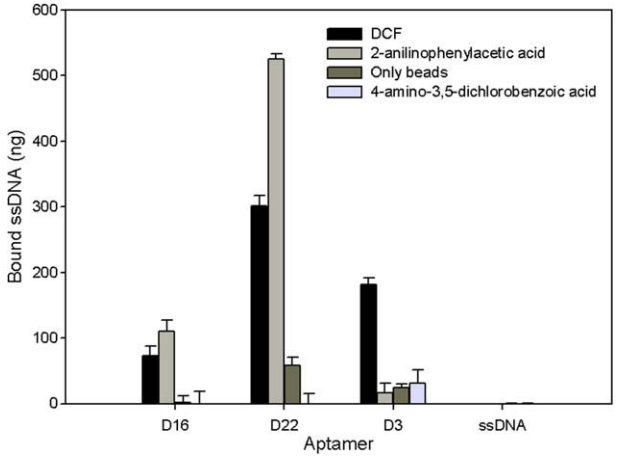

Figure 6. Specificity tests of D16, D22 and D3 aptamers. A constant number of equimolar target coated beads were allowed to bind with 500 nM ssDNA aptamers. The unbound fraction was removed and the traces of unbound or opportunistic bound aptamers were washed and discarded. Bound ssDNA fraction was eluted and the concentration was determined. Aptamers D16 and D22 showed higher affinity for 2-anilinophenylacetic acid than DCF. Only D3 aptamer showed high specificity toward DCF primary target.

moieties. Whereas the D3 aptamer was highly specific to DCF and did not show significant binding to 2APA indicating that D3 preferred binding to the other face of the aromatic ring with two chlorine atoms found specifically on the DCF molecule. Additionally, D3 aptamer showed high affinity to DCF as a target molecule (K_d = 42.7 nM) compared to D22 (166.34 nM) and D16 (148.73 nM) aptamers. The affinity of the aptamers plays an important role in selectivity and specificity of the aptamers to recognize target and its analogs. For example, high affinity of aptamer showed high specificity, such as in case of D3, which binds to DCF but fail to bind its structural analog (2APA) (Fig. 6). Contrastingly, relatively low affinity of D22 and D16 aptamers to DCF was probably be the reason for higher specificity to 2APA than the main DCF target. However, D3 aptamer showed no significant binding to the counter targets which is less than 20% relative to its ability to bind DCF. A different feature of the D10/DA24 aptamer was noticed where the D10/DA24 aptamer although had high affinity toward DCF did not show specificity possibly because of its binding to the most common functional groups or to the aromatic ring on the target molecules. Such aptamers may not be applicable for detection or determining the specificity of a target molecule.

4. Conclusion

Aptamers are single stranded DNA or RNA molecules that can bind to any target from small organic to large protein molecules with high affinity, specificity and selectivity. In this study, a series of potential aptamer candidates (56 aptamers) were selected that bind to DCF, a drug has analgesic, anti-inflammatory and antipyretic properties. Among these 56 aptamers, six of them were selected (D4a, D10/DA24, D16, D22, D30, and D3) based on their sequence and structural similarities and clustering analysis of 40 nt random regions. Out of these six aptamers, only four were found to be potentially high affinity aptamers with K_d values 100.64, 166.34, 148.73, and 42.7 nM for D10/DA24, D22, D16, and D3 aptamers, respectively. Specificity of these aptamers was tested using DCF as the primary target molecule to ascertain that the aptamers selected are specific to DCF or they can also bind to structurally similar counter target, such as 2APA as well as a distinct target ADA. D16 and D22 aptamers recognized the basic nucleus of DCF molecule and can preferentially and selectively bind to 2APA (a structurally similar derivate of DCF). The D10/DA24 showed high affinity (K_d = 100.24 nM) but failed to show specificity toward DCF, whereas the D3 aptamer was highly specific to DCF. The aptamers selected in this study can potentially be applied for capturing of contaminated DCF in body fluids, contaminants and water and as a bioreceptor component for the development of DCF specific biosensor.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.06.044.

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