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Efficient Selection of Glycoprotein-Binding DNA Aptamers via Boronate Affinity Monolithic Capillary

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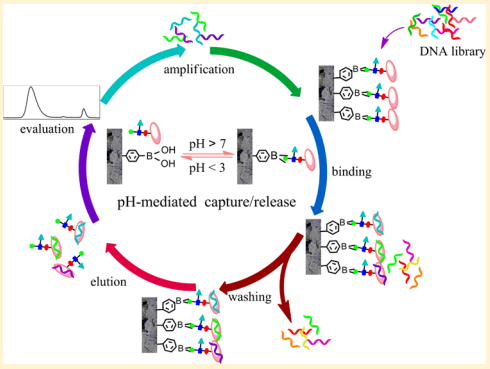
ABSTRACT: Systematic evolution of ligands by exponential enrichment (SELEX) is the workhorse method for selecting aptamers that are capable of binding target molecules from a random oligonucleic acid library. However, conventional SELEX methods are associated with apparent drawbacks including labor-intensive, time-consuming, large reagent consumption and strong nonspecific binding with separation media. Herein, we report a boronate affinity monolithic capillary-based SELEX approach for rapid selection of high-specificity glycoprotein-binding DNA aptamers. Boronate affinity monolithic capillary is an advanced functional material appeared in recent years, which allows for facile capture/release of glycoproteins in a pH-switchable fashion. By using boronate affinity monolithic capillary as a platform for target immobilization and aptamer isolation, the proposed SELEX method allowed for efficient selection of glycoprotein-binding aptamers by 6 rounds and the dissociation constants were at 10^{-8} M level. Because of the employment of boronate affinity monolithic capillary, the new SELEX approach overcame the above-mentioned drawbacks and provided several significant advantages, including rapid selection speed (only 2 days were needed), high specificity toward the target molecules, and minute reagent consumption.



Aptamers are oligonucleic acids or peptides that can specifically bind a target molecule.[1−4] Compared with antibodies, aptamers exhibit advantageous features:[5,6] comparable affinity, easy in vitro synthesis, applicable for a broad range of target molecule (from small inorganic molecules[7] to cells[8]), easy tagging with other functionalities, while retaining original binding ability and much better stability. Nucleic acid aptamers are usually selected from a large random oligonucleic acid pool by systematic evolution of ligands by exponential enrichment (SELEX).[1,4] A key step in SELEX is to isolate target-binding single stranded DNA (ssDNA) from the random pool. Widely used SELEX methods mainly rely on affinity chromatography,[1,9] membrane filtration,[10] magnetic beads,[11] capillary electrophoresis (CE),[12−14] and surface plasmon resonance (SPR),[15] though new methods have been continuously emerging, to name a few, non-SELEX[16−18] and on-chip SELEX.[19] The frequently used methods are all associated with apparent drawbacks. Affinity chromatography, magnetic beads, and SPR based methods require tedious procedures to immobilize targets and to recover bound species,[9,20] magnetic beads and filtration based methods suffer strong nonspecific binding toward targets and oligonucleic acids,[20] while filtration based method is limited to large target molecules.[20] Therefore, novel methods that can effectively overcome these drawbacks are of great importance.

Glycoproteins play crucial roles in diverse biological processes, such as molecular recognition, inter- and intracell signaling, immune response, and so on. Clinically importantly, a variety of glycoproteins have been routinely used as disease biomarkers,[21] while more and more glycoproteins have been suggested as potential biomarkers.[22−24] To date, antibodies are still the major biomolecules for the specific recognition of glycoproteins. As aptamers are advantageous competitors to antibodies, efficient selection of glycoprotein-binding aptamers is of great importance.

Monolithic columns,[25,26] which can be described as integrated continuous porous separation media without interparticular voids, are the newest generation of separation media for liquid chromatography (LC). As compared with conventional LC columns, monolithic columns exhibit several advantageous features, including easy fabrication, low back pressure, fast convective mass transfer, versatile surface chemistry for ligands attachment, and ease of miniaturization in channels and capillaries. Monolithic columns have found important applications in many areas, such as separations[27] and proteomics.[28] Although aptamer-modified monolithic columns have been developed for affinity chromatographic separations,[29−31] to the best of our knowledge, monolithic columns have not been used as platforms for aptamer selection to date.

Boronate affinity monolithic capillaries[32−38] are advanced functional materials appeared in recent years, which allow for reversible covalent capture/release of cis-diol-containing compounds, such as glycoproteins, in a pH-switchable fashion (high pH on, low pH off). Although boronate affinity

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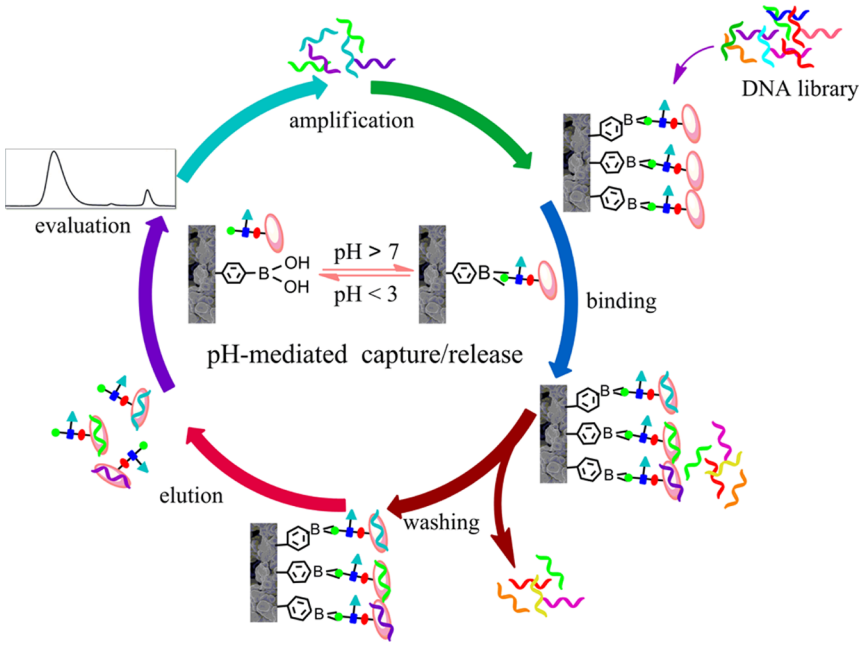

Figure 1. Schematic of boronate affinity monolithic capillary-based SELEX approach.

monolithic columns have shown great promises in chromatographic separations[32−38] and biomimetic materials construction,[39] their strengths in aptamer selection have not been explored yet.

Herein, we report a boronate affinity monolithic capillary-based SELEX approach for efficient selection of glycoprotein-binding DNA aptamers. The key was the employment of boronate affinity monolithic capillary, which allowed for facile immobilization of target glycoprotein and easy recovery of bound species, avoiding tedious procedure and harsh conditions. The principle of the approach is shown in Figure 1. A target glycoprotein dissolved in a protein loading buffer was first continuously injected into a boronate affinity monolithic capillary until all the boronic acid moieties on the monolith were saturated by the target. Unbound target glycoprotein was washed away by the protein loading buffer. Then the monolithic capillary was equilibrated with a DNA loading buffer. After that a random ssDNA library was pumped to the monolithic capillary and incubated with the immobilized target at room temperature for a certain period. Unbound ssDNA species were removed by rinsing the capillary with the DNA loading buffer. Bound species were eluted along with the target by an acetic elution solution and consecutively collected. After evaluation with CE, desired fractions were combined and submitted to PCR amplification. The amplified species were sent for a new round of selection, and the process was repeated again and again until the binding affinity met the requirement or would not increase any more. Because of the employment of boronate affinity monolithic capillary, the approach overcame the major drawbacks existed in conventional SELEX methods. It endowed with rapid selection efficiency (only 6 rounds of selection or 2 days were needed), high specificity toward the target molecules, and minute reagent consumption.

EXPERIMENTAL SECTION

Reagents and Materials. 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES), HRP, myoglobin, and ovalbumin were obtained from Sigma (St. Louis, MO, U.S.A.). Glycine was purchased from Bio-Rad (Hercules, CA, U.S.A.). The DNA library, the PCR primers, and 5(6)-carboxyfluorescein (FAM) were purchased from Sangon (Shanghai, China). TaKaRa Taq enzyme and dNTP were from Takara Biotechnology (Dalian, China). The DNA library was composed of 39 random nucleotides in the central and two constant primer regions (necessary for PCR amplification) at both ends: primer 1, 5′-CTTCTGCCCGCCTCCTTCC-3′; primer 2, 5′-AGTGTCCGCCTATCTCGTCTCC-3′. Other chemical reagents were of analytical grade. The DNA library was dissolved in a DNA loading buffer (20 mM HEPES containing 120 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, and 1 mM $MgCl_2$, pH 7.4). Before selection, the DNA library (100 nM) was heated at 94 °C for 10 min to denature the DNA library and then slowly cooled to room temperature. Proteins were diluted in a protein loading buffer (20 mM HEPES containing 100 mM $MgCl_2$, pH 7.4). All the water used throughout this work was purified by a Milli-Q Advantage A10 system (Millipore, Milford, MA, U.S.A.). CE separation buffer contained 25 mM $Na_2B_4O_7$, 25 mM glycine, and 5 mM KH_2PO_4, pH 9.0, which was filtered through a hydrophilic membrane filter with pore size of 0.45 μm. Fused-silica capillaries of 75 or 150 μm ID × 365 μm OD were purchased from Yongnian Optical Fiber Factory (Hebei, China).

Instruments. CE evaluation and dissociation constant measurements were conducted on a P/ACE MDQ system (Beckman Coulter, Fullerton, CA, U.S.A.), which is equipped with a photo diode array (PDA) detector and a laser-induced fluorescence (LIF) detector. The PDA detection wavelength was set at 214 nm. For LIF detection, the excitation wavelength was 488 nm, whereas the emission wavelength was set at 520 nm. PCR amplification was performed on GeneAmp 9700 instrument (ABI, Shanghai, China). Capillary liquid chromatographic (CLC) separations were carried out on an UltiMate 3000 nanoflow LC system (Dionex, Sunnyvale, CA, U.S.A.), which was equipped with a UV absorbance detector. The signal was monitored at 214 nm.

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Synthesis and Characterization of the Boronate Affinity Monolithic Capillary. The 3-carboxybenzoboroxole-functionalized monolithic capillary of 30 cm (effective length) × 150 μm ID was synthesized according to a previously reported method.[38] The boronate affinity monolithic capillary was washed by HAc (0.1M) for 1 h and conditioned with the protein loading buffer for 1.5 h for the first time. HRP (100 nL, 1 mg/mL dissolved in the protein loading buffer) was injected onto the capillary. The mobile phase started from the protein loading buffer and lasted for 20 min, and then was switched to 100 mM HAc solution (0.1 M). To investigate the retention of the DNA library and the selected aptamer on the capillary, 100 nL of the DNA library, selected aptamer (100 nM, dissolved in the DNA loading buffer), and 1% DMSO (v/v) (dead time marker) were injected separately and the other procedure was the same.

Boronate Affinity Monolithic Capillary-Based SELEX. All steps of the selection procedure, including capillary conditioning, target immobilization, washing, DNA loading, and eluting were performed according to a previously reported manual procedure with slight modifications.[40] Briefly, a certain volume of the reagents was transferred to a 200-μL microcentrifugal tube, which was placed in a high-pressure bomb connected to a nitrogen gas cylinder. A boronate affinity monolithic capillary (25 cm ×150 μm ID) was inserted into the bomb. All solutions were pressured into the capillary by applying 3 MPa of nitrogen gas for a desired period. Although the high-pressure bomb is manually controlled, this simple device was advantageous over a CLC system equipped with an autosampler. Because of the reagents were directly contacted with the monolithic capillary and no connecting tube and device were needed, there was no delayed response and no reagent contamination. The injection volumes of targets and DNA library were only 10−20 μL per cycle, thus saving targets than other affinity SELEX methods.

Prior to each selection cycle, the boronate affinity monolithic capillary was first cleaned with 100 mM HAc solution (0.1 M) for 15 min and then conditioned with the protein loading buffer for 15 min. Fifteen microliters of 1 mg/mL HRP (dissolved in the protein loading buffer) was injected into the monolithic capillary for 5 min and unbound HRP was washed away by applying the protein loading buffer for 5 min. Then, 15 μL of the DNA loading buffer was pressured into the capillary for 5 min to change the buffer condition of the capillary. After that 15 μL of library DNA (100 nM, dissolved in the DNA loading buffer) was pushed into the capillary for 5 min and incubated with HRP for an hour at room temperature. The number of the ssDNA sequences pumped through the monolithic capillary was approximately 9.0×10^{11}, while the number of the sequences incubated with HRP on the capillary was estimated to be only 2.1×10^{11}. Unbound ssDNA species were removed from the monolithic capillary by rinsing the capillary with DNA loading buffer for 3 min. Finally, bound species (in HRP-ssDNA complex status) were eluted with 100 mM HAc solution (pH 2.7) and collected in 4 PCR tubes (2 μL each). After evaluation in terms of CE analysis (see below description), desired fractions were combined and submitted to PCR amplification. The amplified species were sent for a new round of selection. The process was repeated again and again, until the binding affinity met the requirement or would not increase any more.

CE Evaluation. Before being submitted to PCR amplification, the eluates in the PCR tubes were examined by CE to ensure the fractions to be sent to amplify contained the HRP-ssDNA complexes. Before the experiments each day, the separation capillary was first flushed with 0.1 M NaOH at 20 psi for 15 min and CE separation buffer for 15 min. The capillary was rinsed with 0.1 M NaOH for 2 min and the CE separation buffer for 2 min at 20 psi before each separation. The potential was 24 kV and the capillary cartridge was kept at 25 °C. 0.1% DMSO was selected as internal standard and was injected after sample injection. If the HRP-ssDNA complexes in different tubes possessed similar migration time, they were merged together and submitted for PCR amplification.

PCR Amplification and ssDNA Formation. All eluates that contained the HRP-ssDNA complexes were amplified by PCR. PCR reagents were added to the eluates to get the concentration 0.2 mM dNTP, 0.025U/μL TaKaRa enzyme, 0.01 μM primer 1, and 0.01 μM primer 2. Amplification conditions were as follows: 5 min at 95 °C; 18 cycle of 30 s at 94 °C, 30 s at 60 °C, 30 s at 72 °C; 5 min at 72 °C. For the dissociation constant measurement and affinity evaluation, the ssDNA under investigation was labeled with FAM during PCR amplification. The PCR amplification conditions were identical as described above except that primer 1 was replaced by FAM-labeled primer 1. Amplified dsDNA was converted to ssDNA by heating them at 94 °C for 10 min and quickly cooled to −20 °C.

Measurements of the Dissociation Constants Betweem Selected Aptamers and the DNA Library with HRP and Other Proteins. Dissociation constant (K_d) and the affinity of ssDNA toward proteins were measured by CE-LIF. The separation buffer and conditions were identical to those for CE evaluation. Samples containing 4 μL of FAM-labeled aptamers (13 nM) were incubated with varying concentrations of HRP for 1 h and analyzed by CE. The peak height of the free aptamer was fit to eq 1 to estimate the dissociation constant

$$\frac{I_0 - I}{I_0} = \frac{a[\mathrm{HRP}]}{K_d + [\mathrm{HRP}]} \quad (1)$$

where I is the peak height of the free ssDNA when HRP was existed, I_0 is the peak height of the ssDNA in the absence of HRP, and a is a constant. The left part of the equation means the fraction of bound ssDNA, thus the fitting curve is a plot of the fraction of bound ssDNA against the concentration of HRP. Nonlinear least-squares regression analysis was performed to estimate K_d (Prism, version 4.00, GraphPad Software, San Diego, CA). The affinities of aptamers or the library DNA to other proteins were also evaluated by plotting the fraction of bound DNA against the concentration of the protein under investigation. The conditions were the same as described above.

Cloning and Sequencing. After six cycles of selection, the selected ssDNA was PCR amplified for 30 cycles using the conditions listed above and frozen into powder by freeze-drying. Then deionized water was added to the powder up to 10 μL. The DNA pool was cloned and sequenced by Takara Biotechnology (Dalian, China).

Interference of Myoglobin and Ovalbumin on the Detection of HRP. The interference of myoglobin or ovalbumin to HRP was evaluated by CE-LIF. The separation buffer and conditions were identical to those for CE evaluation. Six microliters of FAM-labeled aptamers (13 nM) was incubated with 4 μL of 0.1 mg/mL myoglobin or ovalbumin and HRP of increasing concentration for 1 h, respectively. The control group was 6 μL of FAM-labeled aptamers incubated

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with 4 μL of 0.1 mg/mL myoglobin or ovalbumin without the presence of HRP.

RESULTS AND DISCUSSION

In this proof-of-principle work, we used 3-carboxybenzoboroxole-functionalized monolithic capillary as the selection platform

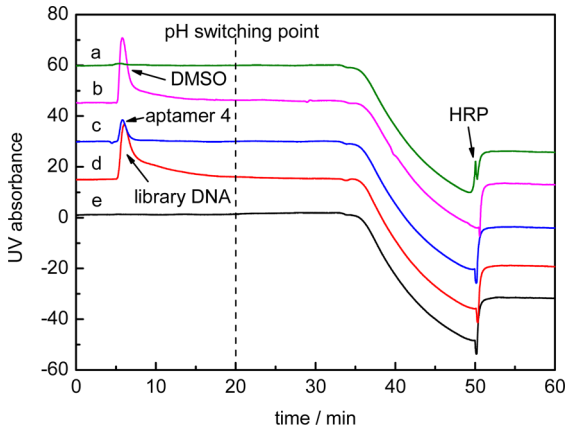

Figure 2. Retention of (a) HRP, (b) DMSO, (c) aptamer 4, (d) the ssDNA library, and (e) blank on the boronate affinity monolithic capillary. Mobile phase: 20 mM HEPES containing 100 mM $MgCl_2$, pH 7.4; switching to 100 mM HAc (pH 2.7) at 20 min.

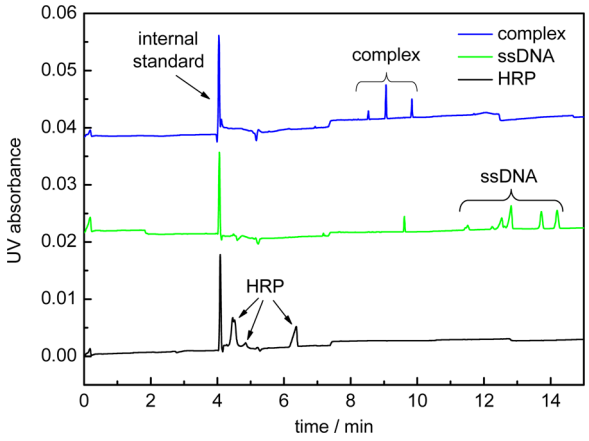

Figure 3. CE electropherograms of (c) HRP standard, (b) the ssDNA selected and amplified from the fourth cycle, and (a) their complexes. Separation buffer: 25 mM $Na_2B_4O_7$, 25 mM glycine and 5 mM KH_2PO_4, pH 9.0. HRP, 1 mg/mL; ssDNA, selected and amplified in the fourth cycle; complex, 1 mg/mL HRP and the ssDNA, incubated for 1 h in boronate affinity monolith column and eluted in fraction.

because of its excellent selectivity and affinity toward glycoproteins at physiological pH.[19] Horseradish peroxidase (HRP), a typical glycoprotein with molecular mass of 44 kDa, was employed as a model glycoprotein. Most of targets in affinity chromatography-based SELEX are small molecules and the immobilization step is tedious. In this study, target glycoproteins could be readily immobilized onto the monolith surface by simply pumping the target molecules into the monolithic capillary at physiological pH, taking only 5 min. Moreover, minute reagent consumption is an additional advantage. The total volume of the monolithic capillary used was only ~3 μL. Because of the limited volume, the current method took only 10−20 μL of target and library DNA per cycle, while traditional affinity chromatography-based SELEX

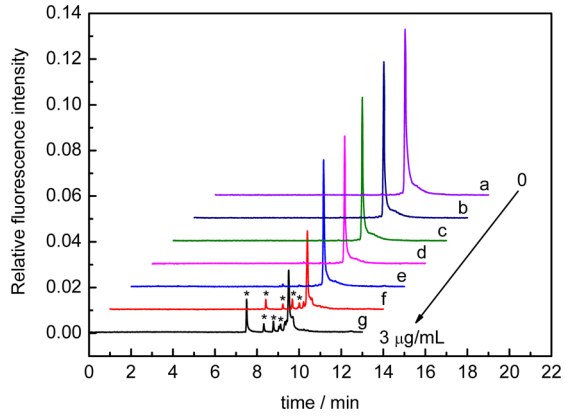

Figure 4. CE electropherograms of FAM-labeled DNA incubated with different concentration of HRP. Four microliters of FAM-labeled DNA was incubated with (a) 0, (b) 0.1, (c) 0.3, (d) 0.5, (e) 1, (f) 2, and (g) 3 μg/mL HRP for 1 h and then analyzed by CE-LIF. Separation buffer: 25 mM $Na_2B_4O_7$, 25 mM glycine and 5 mM KH_2PO_4, pH 9.0. *HRP-DNA complex.

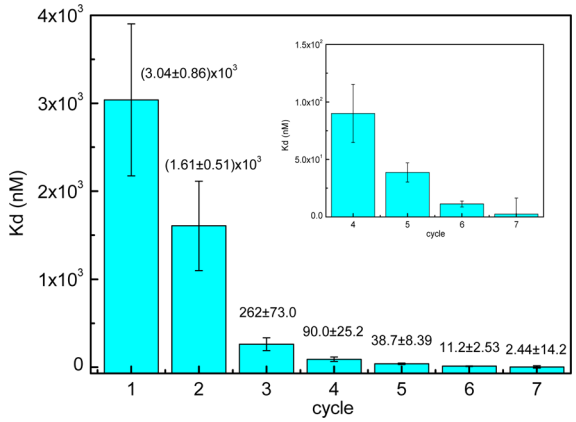

Figure 5. Bulk K_d values for the ssDNA selected at each cycle.

needs several milliliters. Thus this method is very important when the target is expensive or rare.

Selectivity of the selected aptamers is one of the major concerns in aptamer selection. To avoid selection resulted from nonspecific interaction between the ssDNA and the monolith column, the selectivity of the boronate affinity monolithic capillary toward the target and against the ssDNA is very critical. The retention of the target glycoprotein and the DNA pool on the boronate affinity monolithic capillary was first evaluated. As shown in Figure 2, HRP was captured by the capillary at physiological pH but released at an acidic pH (2.7). As a comparison, the DNA library and DMSO were directly washed out of the capillary by the physiological pH buffer. DMSO could not be retained on the surface of the monolithic column, which was set as a dead time marker. DMSO had nearly the same elution time as DNA library, thus the DNA library had no affinity with the monolithic column. The same procedure was also applied to the selected aptamer (aptamer 4). Aptamer 4 exhibited the same elution time as DMSO, so it had also no affinity with the monolithic column. Therefore, the ssDNA selected by the present method can provide high specificity toward the target glycoprotein since only target-binding aptamers were selected.

To ensure only target-bound ssDNA species were submitted for amplification and next-cycle selection, a CE evaluation step

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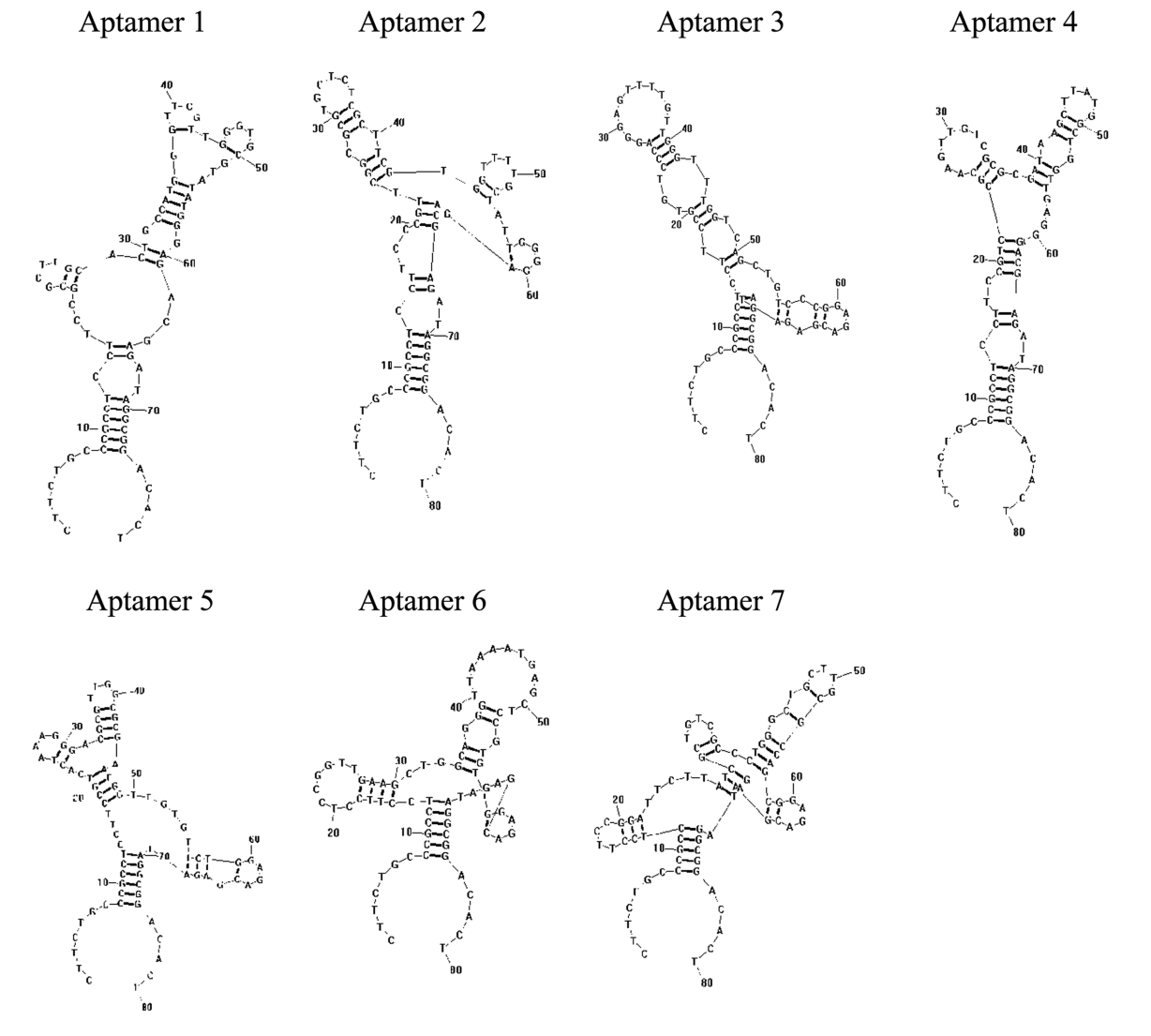
Table 1. Dissociation Constants of the Selected Aptamers[a]

aptamer no.	sequence of random region (5′−3′)	K_d (nM)
1	GCGCTTGCACTGCCATGGGTTCGTTGGGTGCGTATATG	16.2 ± 4.0
2	CGTTCGGCGCGTGCTCTCGCTTCGTGGTTTTGCTATTGG	–
3	GTGTCCCAGGGAGTTTTGTTGGGTTTGGTCAGCTGTCCC	29.2 ± 23.7
4	GTCCGCAAGTTGTCGCGCGATAAGCTTATGGCTGGTTGA	5.9 ± 4.9
5	GTCACTAAAGGGACGCGTTGGCGCGAACGGTTGTGTTCT	7.7 ± 1.7
6	TCCGGTTGAAGCTGGCAGGGTTAAAATGAGCTCCGTGTT	–
7	GGATTCTTATCGCTGTCGCCCTGGGCTGCTTGCGCCAGC	–

[a]—: Not measured.

Table 2. Secondary Structure of the Selected Aptamers



was incorporated in the proposed approach. As shown in Figure 3, free HRP, free ssDNA, and HRP-ssDNA complexes eluted from the boronate affinity monolithic column could be differentiated by CE. HRP usually exhibits a number of isoforms. Three peaks were observed by CE in this study. Three peaks were observed for the HRP-DNA complexes. This is attributed to the multiple isoforms of HRP. When selected ssDNA bind with these isoforms, they could form different peaks. These peaks could not be primers, polymerase or dNTPs introduced at PCR amplification. If they were, the same peaks should had been observed in the electropherogram of ssDNA. If the complexes from a fraction exhibited nearly the same migration times as those from other fractions, all these fractions would be combined and submitted for PCR amplification. However, if a fraction contained no HRP-ssDNA complexes, it would be discarded. In the first two rounds, the content of the complexes was too low to be detected, so all fractions have to be amplified. From the third round, the complexes could be readily recognized. The CE evaluation step was important when the selection condition has not been well established. However,

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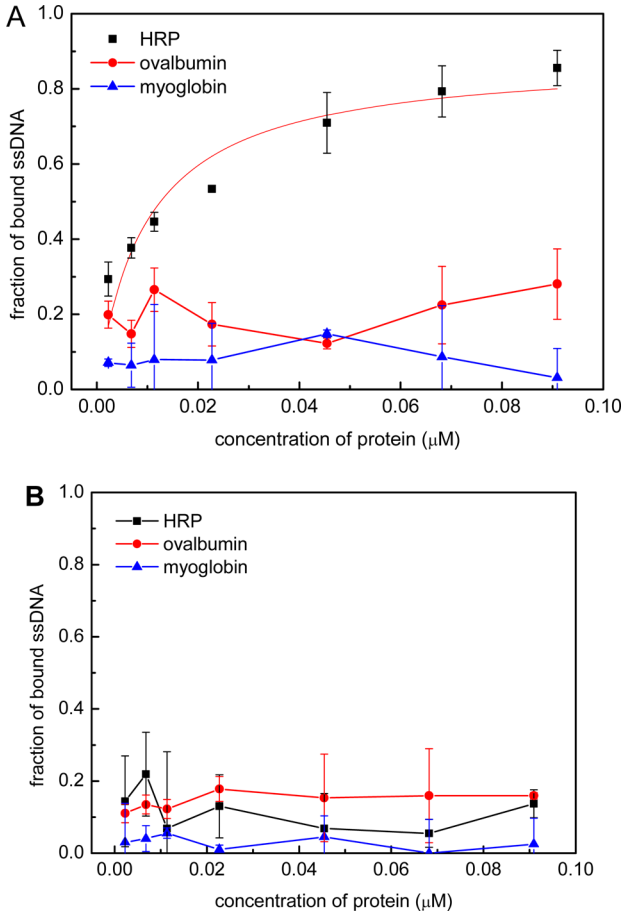

Figure 6. Affinity of aptamer 4 (A) and the DNA library (B) for ovalbumin, HRP, and myoglobin. Four microliters of FAM-labeled DNA was incubated with each protein at different concentrations for 1 h and then analyzed by CE-LIF. Separation buffer: 25 mM $Na_2B_4O_7$, 25 mM glycine, and 5 mM KH_2PO_4, pH 9.0.

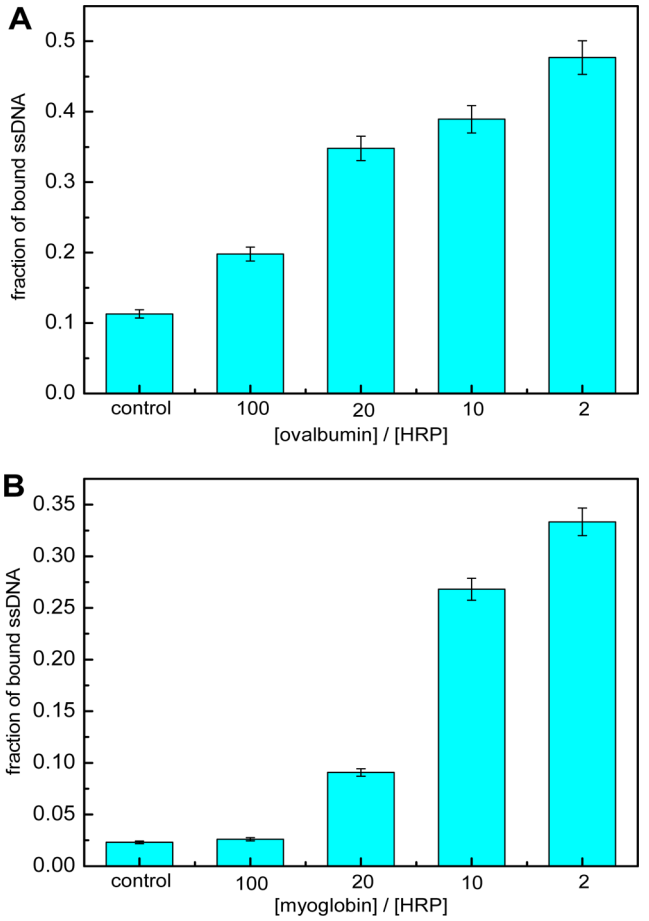

Figure 7. Detection of trace HRP by CE-LIF using FAM-labeled aptamer 4 as a recognition and reporting probe in the presence of myoglobin or ovalbumin. 0.1 mg/mL myoglobin or ovalbumin and HRP of increasing concentration were incubated with FAM-labeled aptamer 4 for 1 h, respectively. Control: 0.1 mg/mL myoglobin or ovalbumin without the presence of HRP.

after the experimental conditions for the selection have been established and well controlled, it could be skipped since the composition of the collected fractions followed nearly the same distribution pattern. For instance, most of HRP-ssDNA complexes were found to exist in the second and third fraction.

The affinity of the selected ssDNA aptamers toward the target after each cycle of selection was evaluated using CE. The electropherograms of the FAM-labeled DNA with increasing concentration of HRP are shown in Figure 4. The height of the FAM-labeled DNA peak decreased as increasing the concentration of HRP. When the HRP reached a relatively high concentration, HRP-DNA complexes were observed. The dissociation constants of the selected ssDNA at 1−7 rounds are shown in Figure 5. The binding strength was exponentially improved as increasing the cycle number in cycles 1−6. After 6 cycles, the dissociation constant (K_d) of the selected DNA toward HRP had reached 11 nM. At the seventh cycle, the dissociation constant did not decrease any more. Although the K_d value was 2.44 nM, the relative error was 14.17 nM thus the actual dissociation constant was nearly the same as that for the sixth cycle. So after 6 cycles, ssDNA aptamers with strong binding affinity to HRP had been obtained. Compared with classical affinity chromatography-based SELEX, the boronate affinity monolithic capillary-based method provided rapid selection speed. Classical affinity chromatography-based method usually takes 8 to 12 cycles or 2−4 weeks to reach dissociation constants at nanomolar level, while the current method took only 6 cycles or 2 days (CE evaluation was included).

The ssDNA obtained at the sixth cycle was cloned and sequenced. Seven aptamers were obtained. Their sequences are listed in Table 1, while their secondary structures are shown in Table 2. Among the seven sequences, four were randomly chosen for the validation of the selection through measuring their K_d values for binding with HRP. The measured K_d values of these aptamers are also listed in Table 1. Since these clones were chosen randomly, they can be considered as a true statistical sampling of the selected pool as a whole. The four representative aptamers exhibited K_d values at nanomolar level, in good agreement with the bulk binding constant at the sixth cycle. These results indicate that the boronate affinity monolithic capillary-based SELEX method was effective.

The affinities of the selected aptamers and the DNA library toward the target, as well as two competing proteins, ovalbumin (glycoprotein) and myoglobin (nonglycoprotein), were evaluated and compared by CE. Aptamer 4 was chosen as a representative of the selected DNA sequences. As shown in Figure 6, aptamer 4 exhibited higher affinity toward HRP as compared with the two competing proteins, while the DNA

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library had nearly the same binding affinity toward the three proteins. Besides, aptamer 4 exhibited significantly higher affinity toward HRP as compared with the DNA library. These results suggest that through the selection, the affinity of the selected aptamer toward the target was effectively improved while the affinity toward the competing proteins was not changed apparently.

The specificity of selected aptamers toward HRP was assessed in terms of their affinities to HRP in the presence of ovalbumin or myoglobin. Aptamer 4 was used for this assessment. Ovalbumin and myoglobin (0.1 mg/mL each) were separately mixed with HRP of different concentrations, and the resultant samples were submitted for CE analysis with FAM-labeled aptamer 4 as a recognition and reporting probe. As shown in Figure 7, aptamer 4 allowed for the detection of 5 μg/mL HRP under the interference of either ovalbumin or myoglobin at 20-fold higher concentration (with a signal 3 times higher than that 0.1 mg/mL ovalbumin or myoglobin produced), indicating the excellent specificity of the selected aptamer under competing environment.

CONCLUSIONS

To conclude, using a boronate affinity monolithic capillary as a general isolation platform, we have established a new SELEX method that allowed for efficient selection of glycoprotein-binding aptamers. The boronate affinity monolithic capillary allowed for facile capture/release of target glycoproteins in a pH-switchable fashion. Because the monolithic capillary allowed for rapid separation, this method demonstrated fast selection speed, taking only 2 days to finish 6 cycles to obtain aptamers with binding constants at 10^{-8} M level. Because of the limited volume of the monolithic capillary, only 10−20 μL of glycoprotein and oligonucleic acid was required for each cycle. As the boronate affinity monolithic capillary did not capture oligonucleic acids, selection resulted from nonspecific binding was avoided. Thus, the proposed method effectively overcame the major drawbacks of existing SELEX methods. Moreover, if combined with an appropriate means that can convert nonglycoproteins into glycoproteins, for example, enzymatic or nonenzymatic glycosylation, this approach could also be applicable to nonglycoproteins. This paper may provide a starting point for rapid development and promising applications of monolithic columns in the aptamer selection area.

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Notes

The authors declare no competing financial interest.

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