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Anal. Chem., **Just Accepted Manuscript** • Publication Date (Web): 10 Jun 2014

Screening of DNA Aptamers against Myoglobin Using Positive and Negative Selection Units Integrated Microfluidic Chip and its Biosensing Application

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ABSTRACT: An aptamer screening method using positive and negative selection units integrated microfluidic chip was introduced. Here, myoglobin (Myo), one of the early markers to increase after acute myocardial infarction, was used as the model. After 7-round selection, the aptamers, which exhibited dissociation constants (K_d) in the nanomolar range (from 4.93 nM to 6.38 nM), were successfully obtained using positive and negative selection units integrated microfluidic chip. The aptamer with the highest affinity (K_d = 4.93 nM) was then used for the fabrication of a label-free supersandwich electrochemical biosensor for Myo detection based on target-induced aptamer displacement. The detection limit of this aptamer based electrochemical biosensor was 10 pM, which was significantly lower than that of those previous antibody-based biosensors for Myo detection. This work may not only develop a strategy for screening aptamer, but also offer promising alternatives to the traditional analytical and immunological methods for Myo detection.

KEYWORDS: Aptamer, Myoglobin, Microfluidic, Selection

Cardiovascular diseases are the one of the leading causes of deaths worldwide. Detecting cardiac markers would be a useful diagnostic tool for cardiovascular disease. Among these cardiac markers, myoglobin (Myo), although not cardiac specific, is one of the very early markers to increase after acute myocardial infarction.[1, 2] Therefore, it is essential to reliably and sensitively detect Myo. At present, some approaches, including electrochemical method,[3, 4] fluorescent method,[5, 6] surface plasmon resonance,[7] and colorimetric method,[8] were developed for Myo detection. Most of these approaches are antibody-based detection methodologies which utilized anti-myoglobin antibody to recognize the target Myo.[3-7] However, the use of antibodies in situ detection methods and in the analyses of complex samples could encounter some limitations mainly deriving from the nature and synthesis of antibodies.[9] The production of antibodies is time-consuming and expensive, and the batch-to-batch variation is a concern.[10] Moreover, the antibodies are sensitive to temperature and undergo irreversible denaturation.[11]

Aptamer, which is often called chemical antibodies, is a good candidate to replace antibodies in therapeutics, diagnostics, and drug development.[12-14] One important advantage for aptamers is that they are more stable and resistant to harsh environment. Unlike antibodies, aptamers can undergo several cycles of denaturation and regeneration. It allows aptamer-based sensing platforms to be recyclable and reusable. Secondly, since aptamer production is completely animal-free, batch-to-batch variations could be avoided and the production of aptamer is very cost-effective and reproducible. Thirdly, aptamers can easily bear labels at each end of the strand. For instance, reporter molecules such as enzymes or fluorophores, were used in the development of aptamer biosensors. Fourth, since aptamers undergo significant conformational changes upon target binding, aptamers offer great flexibility in design of novel biosensors. Generally, protein-binding aptamer sequences have been obtained by using an in vitro process

named Systematic Evolution of Ligands by Exponential enrichment (SELEX).[15-18] However, aptamers for only a few hundred targets have been discovered to date using this method compared with the discovery of thousands of antibodies. This limited success may stem primarily from the iterative nature of SELEX. Particularly relevant here, the whole process of SELEX technology is cumbersome, time-consuming and very expensive.[19] Traditionally, 10-15 rounds selection are required to obtain aptamers with sufficient affinity and specificity.[20, 21] Thus, it is a huge unmet need for faster and less expensive methods of aptamer discovery and improvement.

Some innovative methods have been employed to increase the efficiency and throughput of aptamer isolation, including atomic force microscopy,[22] capillary electrophoresis (CE),[23, 24] boronic acid-based monolith beads,[25] and microfluidic technique.[19, 26-34]. In particular, the integration of microfluidic devices into aptamer screening may provide a valuable key in improving the screening efficiency.[19, 26-34] Some microfluidic incubation, separation and amplification techniques have been investigated as ways to improve the efficiency of aptamer selection, including capillary electrophoresis (CE),[27] sol-gel isolation,[29, 30] magnetic-bead-based selection[31-33] and PCR based on agarose droplet microfluidic technology[34] have been reported. Among most of these previous works based on microfluidic-SELEX, the chips used were generally complicated and needed sophisticated instruments. Thus, the common, convenient microfluidic-SELEX technique which can efficiently and reproducibly generate high performance aptamers is still urgently needed.

Here, a microfluidic-SELEX system with an uncomplicated chip was introduced to screen the aptamer for Myo detection. In this microfluidic-SELEX system, the positive and negative selection units were integrated in the same one channel, thus positive selection and negative

selection could be achieved simultaneously. This integration will simplify the operation process, thus the time can be saved subsequently. Negative selection is important for the specificity of aptamer screening. First, it could avoid the enrichment of unspecific binding oligonucleotides during the aptamer selection process.[35, 36] Second, negative selection is crucial in identifying aptamers which can discriminate between closely related protein targets.[37] The selected aptamer was then used for the construction of highly sensitive and selective electrochemical biosensor for Myo detection based on target-induced aptamer displacement. This work may provide not only an alternative for myoglobin detection but also new strategy for aptamer selection.

EXPERIMENTS SECTION

Materials. Myoglobin protein (from human heart tissue) was purchased from Abcam (USA). C reactive protein (CRP) was purchased from Biovision (USA). Bovine serum albumin (BSA), human serum albumin (HSA) and human immunoglobulin G (IgG) were purchased from Beijing Dingguo Changsheng Biotechnology Co., Ltd. (China). PCR Mix was purchased from Fermentas (Lithuania). Polybead microspheres (25 μm) were purchased from Polysciences (USA). SYBR Gold was purchased from Invitrogen (USA). Hepes and Guanidine isothiocyanate were purchased from Amresco (USA). Hexaammineruthenium (III) chloride ($[Ru(NH_3)_6]^{3+}$, RuHex), 6-mercapto-l-hexanol (MCH) were purchased from Sigma-Aldrich. All of the chemical reagents were of analytical grade or higher. All DNA used in the experiment were synthesized from Sangon Biotechnology (Shanghai) Co., Ltd. (China). The sequences of DNA used here were listed in Table 1. Ultrapure water (18.2 MΩ•cm) was used throughout.

Table 1. Sequences of DNA used

Name	Sequence
Library	5′-FAM-GACAGGCAGGACACCGTAAC-N_{40}-CTGCTACCTCCCTCCTCTTC-3′
forward primer (FP)	5′-GACAGGCAGGACACCGTAAC-3′
reverse primer (RP)	5′-GAAGAGGAGGGAGGTAGCAG-3′
FAM modified forward primer (FP-FAM)	5′-FAM-GACAGGCAGGACACCGTAAC-3′
biotin modified reverse primer (RP-Biotin)	5′-Biotin-GAAGAGGAGGGAGGTAGCAG-3′
forward primer complement (FP-C)	5′-GTTACGGTGTCCTGCCTGTC-3′
capture DNA	5'-HS-TTTTTTTCGGAACAACGC- 3'
report DNA1	5'-ACGTCGAAGGAAAGGAGGGATAAAAGTCAGAAGTCAGAG-3'
report DNA2	5'-CCTCCTTTCCTTCGACGTAGCTCTGACTTCTGACTTTAT-3'

Design and Fabrication of Microfluidic Chip. The microfluidic device was fabricated on a 1.6-mm-thick borosilicate glass substrates using photolithographic and wet chemical etching techniques. It consisted of main straight channel (200 μm wide, 70 μm deep), which included two pinches (1 mm long, 30 μm wide, 15 μm deep), and one branch channel (200 μm wide, 70 μm high) (Figure S1). One pinch, which blocked negative selection proteins coated microbeads, was used as the negative selection unit; another pinch, which blocked targets coated microbeads, was used as the positive selection unit. Type SG3006 glass substrate with 145 nm-thick chromium and 570 nm-thick S-1805 photoresist coating and glass cover plates of the same material were obtained from Shaoguang Microeletronics Corp (Changsha, China). Production of the photomask and procedures for fabrication of microchannels were described below. Briefly, a design on a photomask with microchannels was first transferred onto the glass substrate following a UV exposure. The microchannels were generated in the glass substrate in a well-stirred bath containing dilute $HF/NH_4F/HNO_3$ by twice etching. The narrow channels (i.e. two pinches) were protected by 1 mm wide adhesive tape before the first etching step. Next, the protected adhesive tape was torn out and the glass substrate was etched again under the same condition. The cover, which included three 0.5 mm diameter reservoirs, was bonded to the glass substrate by room-temperature bonding process.[38] This microfluidic chip can be used repeatedly.

Aptamer Selection. The working principle of our positive and negative selection units integrated microfluidic chip for aptamer screening was schematically demonstrated in Figure 1. The target protein (Myo) and negative selection proteins (i.e. HSA, BSA, and CRP) were first incubating with polystyrene microbeads, respectively. The proteins can be easily coupled to polystyrene microbeads via passive adsorption according to the operational manual of Polybead® microspheres. Since the concentrations of proteins in solution can be determined by UV-visible

spectrophotometry, we can estimate the effectiveness of this coating procedure by monitoring the change of absorbance before and after the immobilization of proteins on microbeads. Then protein-coated beads were blocked for 1 hour with 0.1 mM random short ssDNA (20nt). Next, the prepared beads were inhaled into the channel used the pump and blocked before two pinches, respectively (Figure S2 a, b).

A random ssDNA library (~10^{14} random molecules) was used which consisted of a central random region of 40 nucleotides flanked by two fixed regions of 20 nucleotides sequences at the 5' and 3' ends. Then the cDNAs of both primer binding regions, i.e., biotinylated RP and FP-C, were added to binding buffer (20 mM Hepes, 120 mM NaCl, 5 mM KCl, 1mM $CaCl_2$, 1 mM $MgCl_2$, pH 7.3) during DNA library folding by heat treatment. Next, this mixture was carried out at 95 °C for 10 min, and then it was gradually cooled to room temperature. Since the DNA library was passed first negative selection units may sacrify the diversity of DNA library, the amount of DNA library (1340 pmol) in the first cycle was at least 13 times larger than that in the subsequent cycles (100 ~ 60 pmol).

Then, the prepared library was injected into channel at 0.5 μL/min from the inlet 1 (shown in Figure S2a). 120 μL washing buffer (binding buffer, 0.05% Tween20) and eluting buffer (20 mM Tris-HCl, 4 M guanidine isothiocyanate, 1 mM DTT, pH 8.3) were pumped into channel at 2 μL/min from inlet 2 in turn (shown in Figure S2b). Finally, the eluted ssDNA was collected from the outlet (Figure S2 c, d). The processes of selection were monitored by fluorescence microscope (Leica).

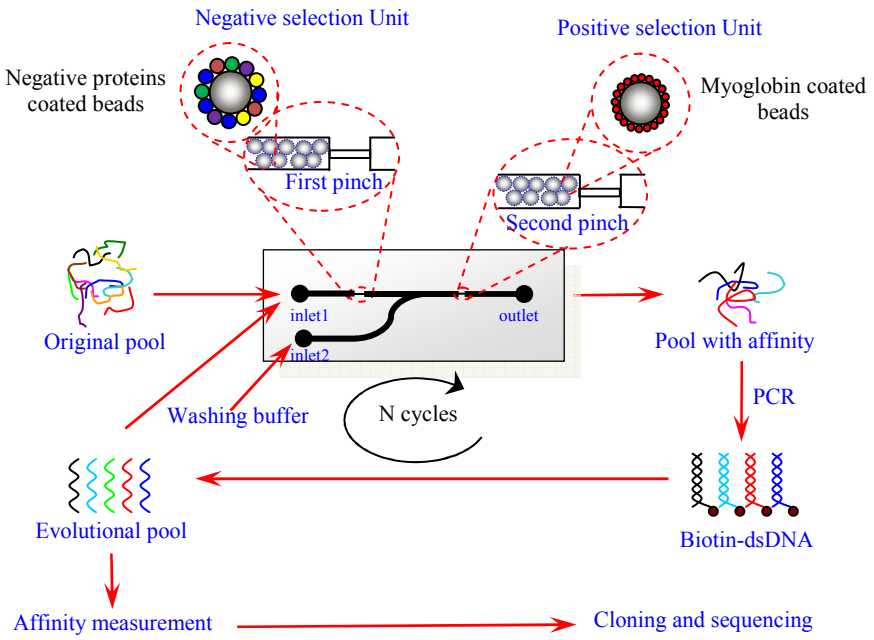


Figure 1. Myoglobin-aptamer selection based on positive and negative selection units integrated microfluidic chip

PCR amplification. Collected products of each round selection were amplified immediately. 5 μL of the collected products were added into a 50 μL PCR mixture with 25 μL 2 × Taq Master Mix, 1 μL of 25 μM FAM-labeled FP, and 1 μL of 25 μM biotinylated RP. The thermal cycling conditions were as follows: 94 °C for 5 min, 10 cycles of three-step PCR (94 °C for 30 sec, 60.5 °C for 30 sec, and 72 °C for 30 sec), terminated by an extra extension at 72 °C for 5 min. Then the amplified PCR products were used as template and diluted to 500 μL in the same PCR mixture. These mixtures were re-amplified. During the extension step of each two cycle, 10 μL of PCR mixture were collected and resolved on 3% agarose gel to find the optimal PCR amplification cycle number. Next, the remaining collected products were all amplified at the optimized cycle number.

Cloning and Sequencing of Selected Aptamers. After 8 rounds of selection, the 6^{th}, 7^{th}, 8^{th} selected products were PCR amplified with unlabeled primers, respectively. The PCR products of each round were sent to Sangon Biotechnology Co., Ltd. (Shanghai, China) for cloning. Hundred colonies of every round were randomly picked and sequenced at the Sangon (Shanghai, China). The secondary structure analysis of selected aptamers was performed with the internet tool Mfold http://frontend. Bioinfo. Rpi. edu/ applications/ mfold/ cgi-bin/ dna-form 1. cgi).

Binding Affinity Evaluation for Selected Products of Each Cycle. To monitor the enrichment of aptamers, binding affinity of each round selected pool was determined using home-made surface plasmon resonance (SPR) instrument.[39] In brief, 10 μM of Myo was first immobilized on uncoated Au film. After blocked by 1 μM random short ssDNA (20 nt), 200 nM unlabeled ssDNA pool (original pool and the 1st-8th selection pool, respectively) dissolved in binding buffer was injected into the flow cell in turn. Each sample was incubated for 30 min and then washed with binding buffer until the signal kept stable. The wavelength shift of SPR for each sample was recorded.

K_d Determination and Selectivity Test of Potential Aptamers. The dissociation constants (K_d) of individual ssDNA aptamers were measured using BIAcore X system (GE Healthcare). The biotin-labeled BSA was first modified on the Au surface at a constant flow rate of 5 μL / min at 25 ^oC. Then 0.05% (w/v) avidin was injected into the channel at the same condition. Next, the biotin-labeled aptamer was injected at a constant flow rate of 10 μL/min. After immobilization of the aptamer, a series of concentration of the Myo was injected at a constant flow rate of 10 μL / min at 25 ^oC in turn and then washing with binding buffer repeatedly. The change of signal (i.e. Response Unit (RU)) was recorded and analyzed.

For investigating the specificity of selected aptamers to Myo, HSA, BSA, CRP and IgG were chosen as control. 500 nM HSA, BSA, CRP, IgG and Myo was introduced at a constant flow rate of 5 μL/min, respectively, and the change of signal (i.e. Response Unit (RU)) was recorded.

Surface Modification of Au Electrodes. Au electrodes was first cleaned in piranha solution (H_2SO_4 / H_2O_2, 3:1(v / v)), followed by a thorough rinsing with water. Then, Au film was modified with the mixture of 2 μM thiolated capture DNA and 2 μM aptamer for 24 h at 4 ^oC, followed by a thorough rinsing with 10 mM PBS (pH 7.4). After 20 min of incubation with 1 mM MCH, Au film was washed thoroughly using 10 mM PBS and the electrochemical sensor chip was obtained. The surface density of capture probe on the Au electrode was measured by chronocoulometry which was performed with a CHI660C electrochemical workstation (Shanghai Chenhua Equipment, China).[40, 41] The conventional three-electrode system was employed, which consisted of Au working electrode, platinum wire auxiliary electrode, and KCl saturated calomel reference electrode. Chronocoulometry was carried out at a pulse period of 250 ms and pulse width of 500 mV. The electrolyte was 10 mM Tris-HCl buffer (pH 7.4) containing 50 μM $[Ru(NH_3)_6]^{3+}$.

Detection of Myo using Electrochemical Aptamer-based Biosensor. Detection of Myo was conducted by exposing the electrochemical sensor chip to different concentration of Myo for reacting about 60 min at room temperature, then washing with 10 mM PBS repeatedly. Next, the mixture of 2 μM report DNA1 and 2 μM report DNA2 was added and incubated for 90 min at room temperature, and then the Au electrode was washed repeatedly with 10 mM PBS. The chronocoulometric signal before and after Myo treatment was recorded respectively. Hot water (85 ^oC) was used to regenerate the Au electrode for further use.

For identifying the target-specificity of this sensing system, four proteins, i.e. HSA, BSA, CRP and IgG were respectively reacted with capture DNA which modified on Au film for 60 min at room temperature, then washing with 10 mM PBS thoroughly. Next, the mixture of 2 μM report DNA1 and 2 μM report DNA2 was added and incubated for 90 min at room temperature, and then the Au electrode was rinsed repeatedly with 10 mM PBS. The change of chronocoulometric signal was recorded.

RESULTS AND DISCUSSION

Myo Aptamer Selection.

The enrichment of the library with Myo-specific aptamers was first monitored using SPR technology. As shown in Figure S3, the resonance wavelength shift of ssDNA specific to Myo was significantly increased with increasing the SELEX round. From 1st round to 5th round selection, the wavelength shift was increased gradually. The signal surged in the 6th round and reached a plateau in the next two rounds, so the 6^{th}, 7^{th} and 8^{th} round selection were cloned and sequenced.

The products from the 6^{th}, 7^{th} and 8^{th} round of aptamer selection were sequenced to reveal the selected Myo-binding aptamers. Among the positive bacterial colonies selected for sequencing, 94, 89 and 109 sequences were picked up, respectively. Among the 6^{th} colonies, eight sequences repeated twice, and a sequence repeated triple. Among the 7^{th} colonies, seven sequences repeated twice, a sequence repeated triple, and a sequence repeated fivefold. Among the 8^{th} colonies, three sequences repeated twice, and a sequence repeated triple. It demonstrated that the selected products of both the 6th and 7th round showed a relatively good enrichment and the enrichment of the 8th round got lower. This result also corresponded with Figure S3.

The homology of the twenty sequences repeated have been analyzed using the software DNAMAN 6.0 into two groups (Table 2). Group 1 and Group 2, which included eight sequences and ten sequences respectively, were significantly homologous to each other with a percent identity of 46.09% and 41.34%. The Myo40-7-34, Myo40-7-69, Myo-40-7-27 and Myo-40-7-23 which have been repeated most times, were selected for further study.

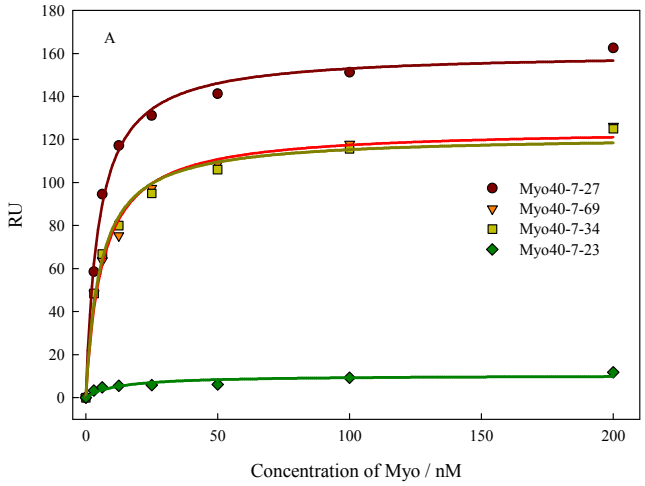
Table 2. Sequences shown in total 3 rounds

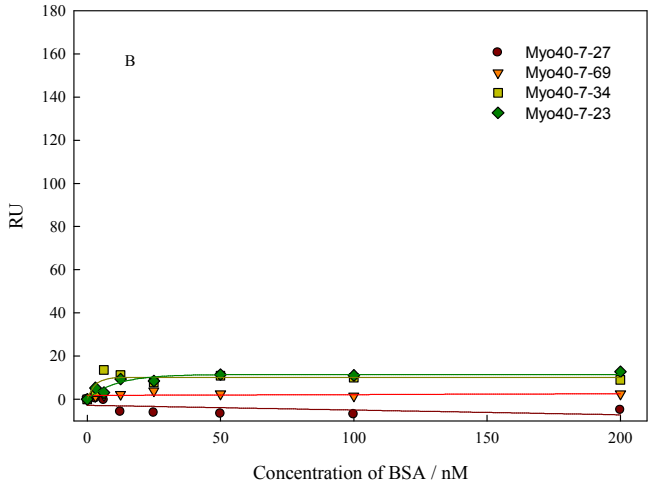
Aptamer ID	Sequences from 5'→3'	Repeat times
Group1 Consistency= 46.09 %		
Myo40-7-27	------CCCTCCTTTCCTTCGACTTAGATCTGCTGCGTTGTTCCGA--	4
Myo40-6-56	--TATCGCCACCGTGCTCTCTTCTTCGATTCTCTGTCTCGTC------	2
Myo40-6-43	CAGATTCCCGCCGGCCCATCGTCTCTAGTCGACCGTTTGG--------	2
Myo40-7-10	-------CACCCCTACAACCTACTTCACAACACCGCTTTTTCCGTTC-	2
Myo40-6-42	-----TCATGTAGGTATGGCCCTTTGGTTGTGCCCTCTTTTGGTT---	2
Myo40-7-22	--------GCGCTTTCTAGATGCTTCCACGCACTGTTTTGGTCCCGTA	2
Myo40-6-58	----CCGGCATATTGGTCCGGCTTGTCTTTTCTCGGGAGTGTTT---	2
Myo40-8-12	ACAACTATAGGCATCGTATTTGGCTAATCAATCTGCTTGC-------	2
Group 2 Consistency= 41.34 %		
Myo40-7-34	-------ACGCACAATTCCTTGTCCAATTAGGAAATTCTACGCGGAT--	10
Myo40-7-69	---------CGAGTACTTCTTTGCTAGTTCGCGAGATACGTTGGCTAGG	7
Myo40-7-23	---------GACATCTATCGCCTCGATTTCCTTTGGTGAGATCGGCTCC	3
Myo40-6-40	-------CACCCAGCCCTTCGTACTAGCTAATGTTTTACTGTTTATG--	2
Myo40-6-78	---TCCCGTATGTGAAGTCGCTTTGAACTCGCCTAGTGCGGTG------	2
Myo40-6-36	---------CCGGGCTCTCGCTTCTAAACCCTCTGTTGTCGTGGTACGC	2
Myo40-8-93	----CCGAGACAAACTCTCGGCGCGATGTATATTAACCCCTTCC-----	2
Myo40-7-38	GAATGCGATCCCACAACACTCCGCGCCGTCTGATGGTGTG---------	2
Myo40-7-73	----TGCGTATCCAAATTAAGATCGAGCCGCATGAATCGGGCGC-----	2
Myo40-7-12	--GACGTCTTGGGCATAGCAGCGCCGGCAGCCCATTTTCGGC-------	2

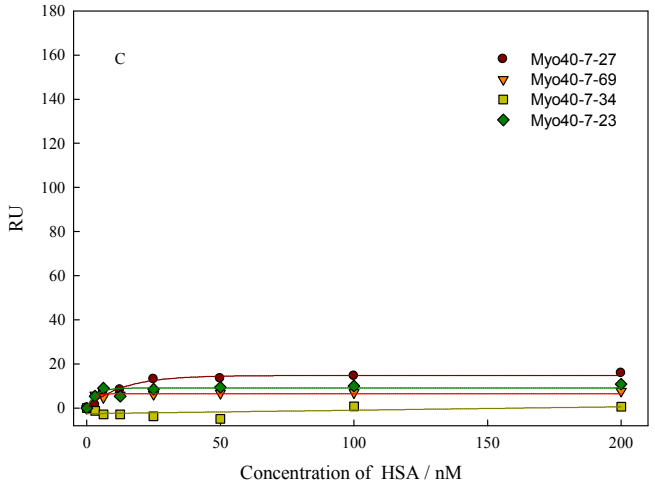
Determination of Affinity and Specificity. The four selected sequences was first immobilized on the Au surface respectively, and then affinity and specificity of the four selected sequences was evaluated by their binding capacity with different concentration of Myo. The aptamers that had the capacity to bind strongly were selected based on the dissociation constant (K_d). To calculate K_d, the SPR response values, i.e. RU versus Myo concentrations was plotted, and the data points were fitted by the non-linear regression analysis.[42, 43] As shown in Figure 2A, Myo40-7-27, Myo40-7-69 and Myo40-7-34 can bind Myo with high affinity (K_d of Myo40-7-27, Myo40-7-69 and Myo40-7-34 was 4.93 ± 0.43 nM, 6.38 ± 0.80 nM and 5.58 ± 0.66 nM, respectively); while Myo40-7-23 and DNA library showed negligible binding affinity to Myo.

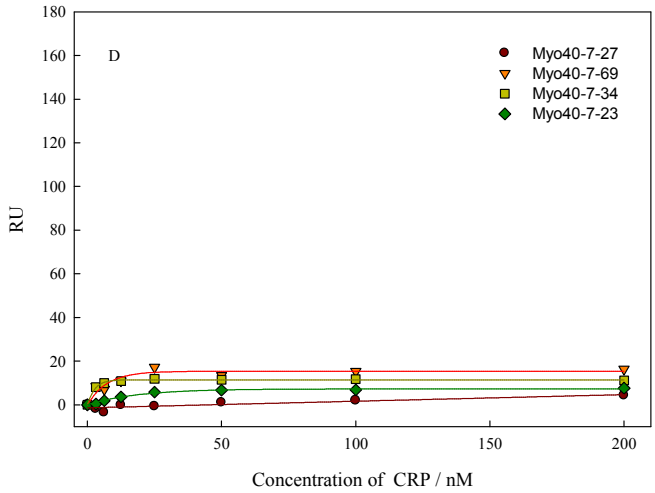
The selectivity of the sequences was then analyzed by comparing their ability to distinguish between Myo and other proteins (such as BSA, HAS, CRP and IgG). As shown in Figure 2B-2E, the results indicated that Myo40-7-27, Myo40-7-69 and Myo40-7-34 selectively signaled the presence of Myo, while the addition of the other four proteins cannot significantly influence the

shift of RU. Similarly, the change of RU which was resulted by Myo40-7-23 was not clear. Especially, among these four sequences, Myo40-7-27 displayed the excellent affinity and selectivity for Myo. The highest affinity to Myo was obtained for aptamer (Myo40-7-27) with a K_d of 4.93 nM and therefore was used for further experiments.









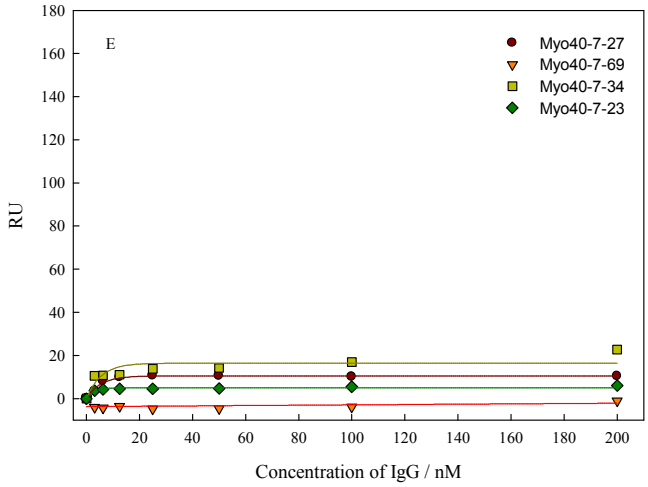


Figure 2. Affinity and specificity determination for selected aptamers. Detection of different

concentration of MYO(A), BSA (B), HSA (C), CRP (D) and IgG (E) using different aptamer.

Electrochemical Aptamer-based biosensor for Myo Detection. The aptamer with the highest affinity to Myo (i.e. Myo40-7-27) was then used to construct a label-free supersandwich electrochemical biosensor based on target-induced aptamer displacement. The scheme was similar to our previous work. [41] As shown in Figure 3, thiol modified capture DNA was first assembled on the Au electrode. Next, as Myo40-7-27 was added, it was then captured on the Au electrode due to DNA hybridization. In the absence of Myo, supersandwich structures could be formed on the Au surface as report DNA1 and report DNA2 were introduced. Since RuHex could bind to anionic phosphate of DNA strands in a stoichiometric approach, redox charge of RuHex was a direct function of the amounts of DNA strands localized at electrode surfaces. As target Myo was introduced, the interaction between aptamer and Myo displaced the aptamer from the Au surface. Even if report DNA1 and report DNA2 were added, the supersandwich structures could not form, resulting in decrease of the amount of RuHex on the electode, i.e. a lowered electrochemical signal. The detection mechanism of this electrochemical biosensor was based on probing the change of redox RuHex charge caused by the Myo-induced aptamer displacement.

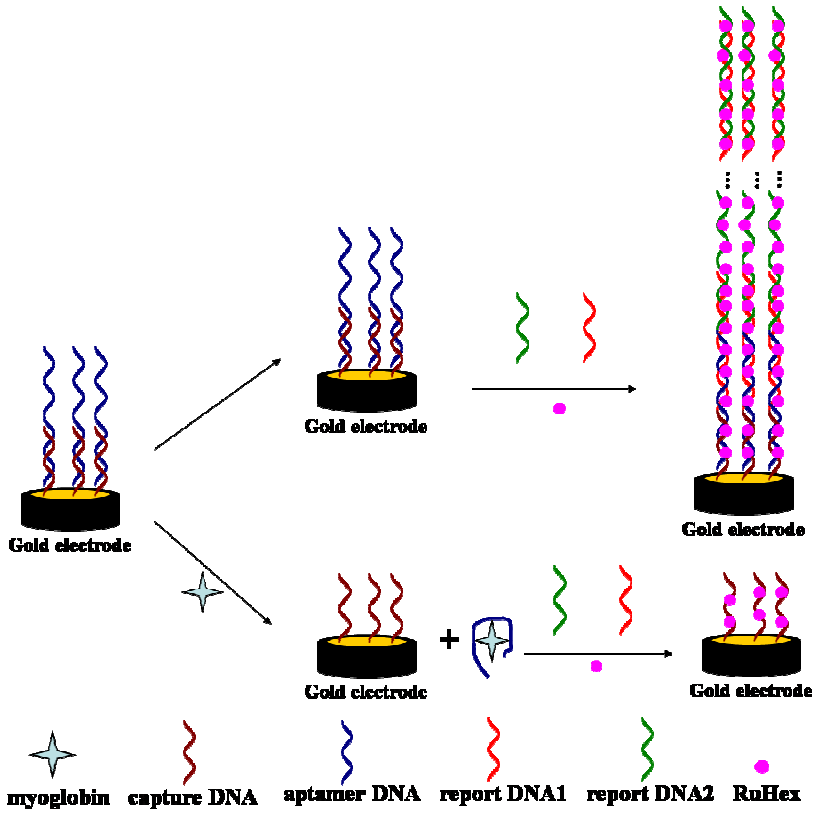


Figure 3. Schematic of electrochemical biosensor for Myo detection with supersandwich amplification strategy.

Some factors influenced the sensitivity of this biosensor. Here, as shown in Figure S4 of Supporting Information, the optimal surface density of capture DNA, aptamer concentration, reaction time of Myo, report DNA concentration, and the formation time of supersandwich structure were 8.8×10^{12} molecule/cm^2, 2 μM, 90 min, 2 μM and 90 min, respectively. Under the optimized conditions, different concentrations of Myo were detected. Figure 4A showed that the redox charges of RuHex gradually decreased as the Myo concentration increased from 10 pM to 100 nM. Figure 4B showed the relation between the decrease of redox RuHex charge (ΔQ and the concentration of Myo. ΔQ = Q1-Q2, Q1 and Q2 were the charges before and after Myo treatment, respectively. ΔQ increased as the Myo concentration increased. According to the 3σ

rule, the detection limit was 10 pM for the supersandwich electrochemical biosensor. This sensitivity is comparable to or better than that of those previous antibody-based biosensors for Myo detection.[3-7, 44]

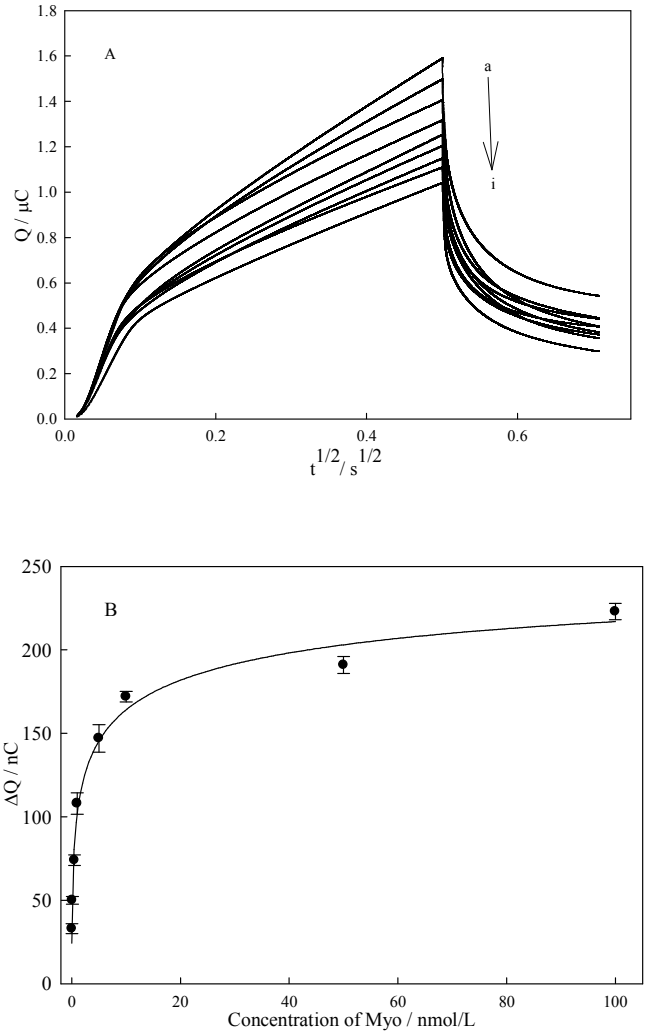


Figure 4. (A) Chronocoulometry curves for Myo at different concentrations. From (a) to (i), it presented the chronocoulometry curves of binding with Myo at concentrations of (a) 0, (b) 10 pM, (c) 0.1 nM, (d) 0.5 nM, (e) 1 nM, (f) 5 nM, (g) 10 nM, (h) 50 nM, and (i) 100 nM. (B) Detection of different concentrations of Myo based on electrochemical biosensors with supersandwich amplification. Error bars showed the standard deviation of measurement taken

from six experiments. Surface density of capture DNA: 8.8×10^{12} molecule/cm^2; Concentration of Myo40-7-27: 2 μM; Reaction time of Myo on the electrodes: 90 min; Concentration of report DNA: 2 μM; Supersandwich-formation time: 90 min.

Besides sensitivity, the selectivity of this aptamer-based electrochemical biosensor was also investigated. Four proteins, i.e. CRP, BSA, HAS and IgG, were used as contrasts. As shown in Figure 5, for these four proteins, all of ΔQ were lower than 10 nC. It implied that this biosensor showed high selectivity for Myo detection.

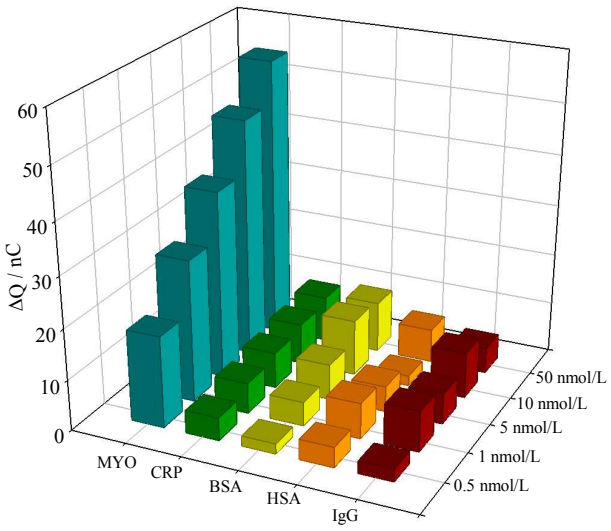


Figure 5. The chronocoulometric response of Myo and other four proteins.

This biosensor was also used to detect Myo in human serum. The human serum was diluted 100 times using 10 mM PBS. Next, the Myo-spiked serum samples were prepared by adding Myo in diluted serum samples, and then were detected. As shown in Figure S5, the signal caused by Myo in human serum was similar to that in buffer, suggesting that the other components of the serum did not interfere significantly in this assay. The excellent result of our method for Myo

detection in human serum demonstrated its potentials for practical applications in disease diagnostics.

CONCLUSION

This work showed the first selection, identification, and biosensing application of DNA aptamers for Myo. Through the positive and negative selection units integrated microfluidic chip, the high affinity aptamers, which exhibited their K_d values at the nanomolar level, were successfully obtained through a 7-round selection. The highest affinity aptamer (K_d = 4.93 nM) showed high sensitivity and selectivity for Myo detection. Based on target-induced aptamer displacement, we developed a limit of detection of 10 pM with a label-free supersandwich electrochemical aptamer-based biosensor. We envision that this positive and negative selection units integrated microfluidic chip may have the potential to be applicable to screening molecules against aptamers. Moreover, this work may provide a potential tool for Myo detection.

ASSOCIATED CONTENT

Supporting Information. Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENT

This work was supported by the National Natural Science Foundation of China (21190040, 21221003, and 21175035), National Basic Research Program (2011CB911002), International Science & Technology Cooperation Program of China (2010DFB30300), and Fundamental Research Funds for the Central Universities.

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