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Target replacement strategy for selection of DNA aptamers against the Fc region of mouse IgG

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ABSTRACT. Aptamers that recognize the IgG Fc region are of great interest because of their wide application as an immunology probing tool, for diagnostics, and as affinity agents for antibody purification. We developed a target replacement strategy as a modification of conventional Systematic Evolution of Ligands by EXponential enrichment (SELEX) in order to efficiently select and identify novel DNA aptamers against the Fc region of mouse IgG. In this new approach, multiple IgG subclasses (IgG1, IgG2a, mouse IgG Fc, and anti-HBs IgG) were sequentially used to select aptamers in one continuous SELEX. After 8 rounds of selection, the aptamers were analyzed using dot blot and an electrophoretic mobility shift assay, which showed universal binding capability to different IgG subclasses. Secondary structure analysis of the aptamers indicated that the stem-loop structure of the aptamers play an important role in binding to the common site in different mouse

IgG subclasses. This demonstrated the feasibility of using multiple target replacement SELEX for the selection of aptamers. This target replacement strategy is also expected to be useful for selecting aptamers that bind common regions of molecules other than antibodies.

Key words: Target replacement; DNA aptamer; Fc region of mouse IgG; SELEX

INTRODUCTION

Apart from their function in storing genetic information, nucleic acids (DNA/RNA) have a long history as affinity molecules (aptamers) that recognize a wide range of targets. The high specificity and affinity of aptamers is based on the ability of DNA and RNA to form intricately folded and unique 3-dimensional structures that interact with their targets. Soon after aptamers were first described as protein binding ligands (Ellington and Szostak, 1990) in 1990, Systematic Evolution of Ligands by EXponential enrichment (SELEX) technology (Tuerk and Gold, 1990) was used for *in vitro* selection and amplification of aptamers. SELEX technology is currently an important and widely used tool in molecular biology, pharmacology, and medical research. The technology has been further developed to accommodate the increasing need for various aptamers against versatile target molecules (Miyakawa et al., 2006; Chu et al., 2006; Shamah et al., 2008; Keefe and Cload, 2008). To ensure high affinity and specificity of selected aptamers, a critical step in the SELEX process is to stringently isolate the bound and unbound DNA after incubation of the nucleic acid library and target. To that end, a variety of modified SELEX selection methods have been proposed to make it a more efficient and less time-consuming process. In the FluMag-SELEX method (Stoltenburg et al., 2005), fluorescent labels are used for DNA quantification and magnetic beads are used for target immobilization. The advantages of this strategy include easy handling, use of very small amounts of target, rapid and efficient separation of bound and unbound molecules, and compatibility to targets with very different properties and sizes. The use of filter immobilization (Hall et al., 2010) in SELEX provides a general way to efficiently separate target bound RNAs for RNA aptamer selection. A remarkable improvement in the resolution of bound and unbound nucleic acids can be obtained with the use of the capillary electrophoresis technique (Mairal et al., 2008) in SELEX, and only a few cycles are required for the isolation to obtain high-affinity aptamers, such as the high-affinity aptamer that binds with human immunoglobulin E (IgE) (Mendonsa and Bowser, 2004). The Photo-SELEX method also results in high-affinity and selectivity aptamers (Mairal et al., 2008). In this process, a photoactivatable nucleotide is incorporated with the nucleic acids, and photo-induced covalent bonds can be formed with the target molecule, thus greatly improving partitioning efficiency. Automated SELEX protocols dramatically shorten the time used in the standard selection process, making it now possible to obtain aptamers within days rather than weeks (Cox and Ellington, 2001; Nitsche et al., 2007).

Antibodies are widely used as a tool in protein identification and quantification. One antibody that is used as a major probing tool is immunoglobulin G (IgG), and aptamers against it have been of great interest due to their broad potential applications. Aptamers specifically recognize the highly conservative fragment, crystallizable (Fc) region in IgGs, thus providing a general affinity agent for the IgG family, which is highly desirable (Sakai et al., 2008). It can

be used as a conventional secondary antibody, providing a general, simple, and sensitive tool for an ELISA system, or it can also provide a new affinity agent for mass purification of therapeutic antibodies (Sakai et al., 2008; Yoshida et al., 2009). Using the conventional SELEX method, the rabbit antibody was recognized from a pool of 2^{10}-2^{14} RNA aptamers (Yoshida et al., 2008). One of the aptamers displaying binding activity to the rabbit IgG was shown to be a highly universal molecule against a conserved region of IgG. This study suggested that standard SELEX could yield multiple aptamer species against IgG.

Due to its structural complexity, selection of a universal aptamer against the Fc region of IgG remains a challenge and a matter of chance.

To overcome the difficulties in standard SELEX procedures, and to achieve straight forward, well-controlled selection of universal aptamers against the Fc region of IgGs, we propose a modified SELEX method in which different IgG subclasses (anti-HBs IgG, mouse IgG Fc, IgG1, and IgG2a) could serve as binding targets during the selection process. Each of the IgG subclasses could be used sequentially to select bound DNAs, and are replaced every 2 rounds, leading to final aptamers that are capable of binding their common sites to these IgG subclasses (Figure 1). We describe the target replacement strategy modified SELEX procedure and the properties of the selected aptamer against the Fc region.

After 8 rounds of selection, we obtained 49 aptamers with affinities to mouse IgG. Through binding assay by dot blot and electrophoretic mobility shift assay (EMSA) experiments, we successfully selected and identified 2 aptamers with higher affinities and specificities to mouse IgG. This study provides a solid platform for future research related to molecular structure and function.

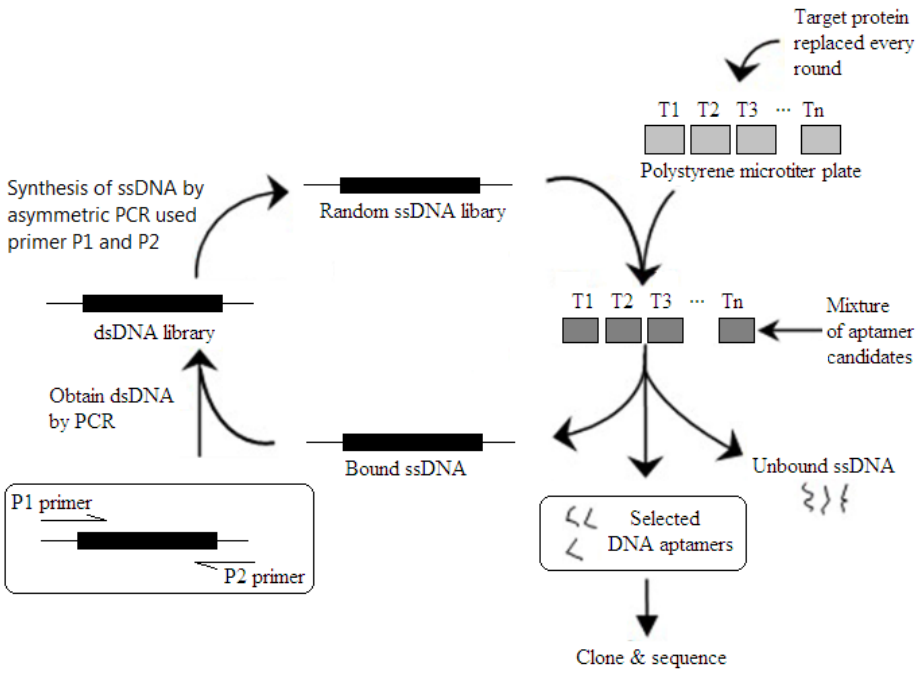

Figure 1. Generalized scheme indicating the key steps in the target replacement SELEX.

MATERIAL AND METHODS

Material

[α-^{32}P]-ATP (specific activity = 3000 Ci/mmol; radiochemical purity = 95%) was purchased from Furui Biotech ^{32}P Lab (Beijing, China). Mouse myeloma IgG1, mouse myeloma IgG2a, mouse IgG Fc fragment, and mouse monoclonal antibody resistant to HBs IgG (5 mg/mL) were all purchased from PIERCE (USA). The pGEM®-T vector and PUC19 plasmid were obtained from Promega Bioscience (USA) and TaKaRa (Dalian, China), respectively. An *Escherichia coli* DH5α-sensitive cell line was prepared by our laboratory, and the transformation ratio was 1.86 x 10^6. Random-library DNAs were constructed and synthesized by SBS Genetech Co. Ltd. (Beijing, China). Primers (P1: 5'-TAA TAC GAC TCA CTA TAG CAA TGG TAC GGT ACT TCC-3'; P2: 5'-TTA GCA AAG TAG CGT GCA CTT TTG-3'; T7: 5'-TAA TAC GAC TCA CTA TAG G-3'; SP6: 5'-ATT TAG GTG ACA CTA TAG AA-3') were all synthesized by Sangon Biotech Co. Ltd. (Shanghai).

Methods

Target replacement SELEX

Four groups of polystyrene microtiter plates (Maxisorp96-wells, Nunc) were coated with anti-HBs IgG, mouse IgG Fc, IgG1, and IgG2a (50 µL, 5 mg/mL) in 0.05 M carbonate buffer, pH 9.6, overnight at 4°C. The same conditions were used to generate the negative control, which was treated with carbonate buffer without IgG. After the wells were blocked with Tris-buffered saline (TBS), containing 5% (w/v) fat-free powder milk (TBSM), the anti-HBs IgG-coated microtiter plates were used for the first 2 rounds of selection. A typical random library (5'-TAA TAC GAC TCA CTA TAG CAA TGG TAC GGT ACT TCC (N x 25) CA AAA GTG CAC GCT ACT TTG CTA A-3'; N = A, C, T, G) was constructed as previously described and denatured by heating at 95°C for 5 min. Then, the mixture was immediately cooled to 0°C in selection buffer [5 mM $MgCl_2$ in phosphate-buffered saline (PBS)]. To reduce background interference, 1 mg/mL yeast tRNA (Sigma) was added. The selection was performed by washing 3 times to elute the IgG-aptamer complexes with buffer (7 M urea, 0.5 M NH_4Ac, 1 mM EDTA, and 0.2% SDS), and extracted with phenol-chloroform. The ssDNA was precipitated with 10 mM $MgCl_2$ and 0.3 M NaAc, as well as a 2.5-fold volume ethanol. Selected ssDNA was dissolved in PCR mixture containing 10 µL 10X PCR buffer, 8 µL dNTP (10 mM each, Takara), 6 µL 25 mM $MgCl_2$, 1 µL 25 mM P1 long primer (5'-TAA TAC GAC TCA CTA TAG CAA TGG TAC GGT ACT TCC-3'), 1 µL 25 mM P2 primer (5'-TTA GCA AAG TAG CGT GCA CTT TTG-3'), 73.5 µL water, and 0.5 µL (2.5 U) Taq DNA polymerase (Takara). The mixture was thermally cycled 5 times at 94°C for 1 min, at 37°C for 2 min, and at 72°C for 2 min. The purified PCR products were passed onto the next 2 rounds of SELEX using mouse IgG Fc-coated microtiter plates as the binding platform to further select and amplify bound DNAs. This process was repeated for IgG1 and IgG2a for 2 rounds each, for 8 rounds of selection.

Preparation of ssDNA aptamer and radioactive labeling

The PCR mixture, consisting of 5 µL 1 pM ssDNA templates, purified as described above, 2 µL 25 mM P1 primer, 0.2 µL 25 mM P2 primer, 8 µL dNTP (10 mM each), 6 µL 25 mM $MgCl_2$, 10 µL 10X PCR buffer, 68.3 µL water, and 0.5 µL (2.5 U) Taq DNA polymerase (Takara), was thermally cycled (94°C for 30 s, 55°C for 30 s, 72°C for 30 s, for 35 cycles). The PCR product was purified on 10% denatured polyacrylamide (0.5% bisacrylamide) gels containing 7 M urea. The radiolabeled ssDNA aptamer was prepared using the same method described above, except that [^{32}P]-dATP was added to the PCR mixture instead of dATP.

Binding assay by dot blot

Mouse IgG subtypes (6 µg anti-HBs IgG, 6 µg mouse IgG Fc, 6 µg IgG1, and 6 µg IgG2a) were dot blotted on nitrocellulose membranes and blocked at 37°C for 30 min with TBS containing 5% (w/v) TBSM. Similarly, 6 µg trypsin, 6 µg solcoseryl albumin, and 6 µL human serum were also dot blotted on nitrocellulose membranes and blocked as described above. The ssDNAs selected from 2, 4, 6, and 8 rounds of SELEX, from both negative and target wells, were radiolabeled as previously described. They were then denatured at 95°C and immediately cooled to 4°C in selection buffer (5 mM $MgCl_2$ in PBS). The dot blotted membranes were then incubated with an equal amount of radiolabeled ssDNA aptamers mixed in 200 µL selection buffer at 37°C for 30 min. After washing 3 times with 3 mL selection buffer, the nitrocellulose membranes were air-dried and autoradiographed. The blots were further analyzed using the 2-D Gel Image Analysis Software Package (Melanie 3).

Selection of positive transformed clones

The ssDNA aptamers, prepared as described above, were transformed into *E. coli* using the pGEM®-T vector system (Promega). Plasmids were separated from single bacterial colonies. Interlace PCR, with consensus and specific primers, was used to select positive clones.

Two groups of PCR mixtures were prepared, in which each 10-µL mixture contained 0.08 µL dNTP (10 mM each; Takara), 0.6 µL 25 mM $MgCl_2$, 1 µL 10X PCR buffer, 7.1 µL water, 0.1 µL (2.5 U) Taq DNA polymerase (Takara), 0.2 µL consensus primer T7 (25 mM), and 0.2 µL specific primer P1 (25 mM) for the group 1 mixture, or 0.2 µL specific primer P2 (25 mM) for the group 2 mixture. Thereafter, half of a clone was identified in a tube using the group 1 mixture, while the group 2 mixture was used in another tube. The tubes were thermally cycled (94°C for 30 s, 55°C for 30 s, 72°C for 30 s, for 35 cycles). After PCR, the products were subjected to 10% non-denatured polyacrylamide gel electrophoresis (PAGE) separation and the positive clones were further sequenced (SBS Genetech Co. Ltd).

EMSA

After ssDNA aptamers were labeled by [α-^{32}P]-ATP as described above, 100 pM [^{32}P]-labeled DNA aptamers was incubated with serial dilutions of IgG (from 5 to 20 µg) in 20 µL binding buffer (10 mM HEPES, pH 7.6, 1 mM EDTA, 2 mM dithiothreitol, 50 mM KCl, 0.05% Triton X-100, and 5% glycerol) at 37°C for 1 h. Bound and unbound DNA species

were separated on a 0.5% native agarose gel in 0.5X Tris-borate-EDTA electrophoretic buffer at 80 V for 2 h. The gel was then autoradiographed after being fixed for 2 h with 10% acetic acid and 50% methanol and dried. After autoradiography, the same gel was stained again with Coomassie brilliant blue, eluted, and photographed.

RESULTS

Target replacement-modified SELEX

In order to obtain aptamers that could universally recognize a general range of IgG members, a target replacement strategy to modify standard SELEX was used. In this new approach, multiple-IgG subclass targets were sequentially used and replaced by another in one SELEX. After using a different IgG subclass target in the next round of selection, only the aptamer candidates able to bind to a site common to the 2 IgG subclasses were able to pass on to the subsequent selection round. Therefore, consecutive replacement of the 4 IgG subclasses would ultimately generate universal aptamers that could bind to all IgG subclasses. In this study, anti-HBs IgG, mouse IgG Fc, IgG1, and IgG2a were used to test the feasibility of this new approach.

Target binding ability of the selected aptamer candidates was evaluated by dot blotting and radioactivity intensity analyses using the Image J Application software. The radioactivity intensity results were 10.9, 22.0, 27.5, and 36.5 after 2, 4, 6, and 8 rounds of selection, respectively; however, the subtractive binding intensity results were 8.1, 8.9, 10.8, and 11.1. Compared with the subtractive group, the aptamer target binding ability rose smoothly after every 2 rounds of selection upon replacement of the IgG subclass target. These results (Figure 2) demonstrate that we successfully obtained an aptamer library with high specificity and affinity after 8 rounds of selection.

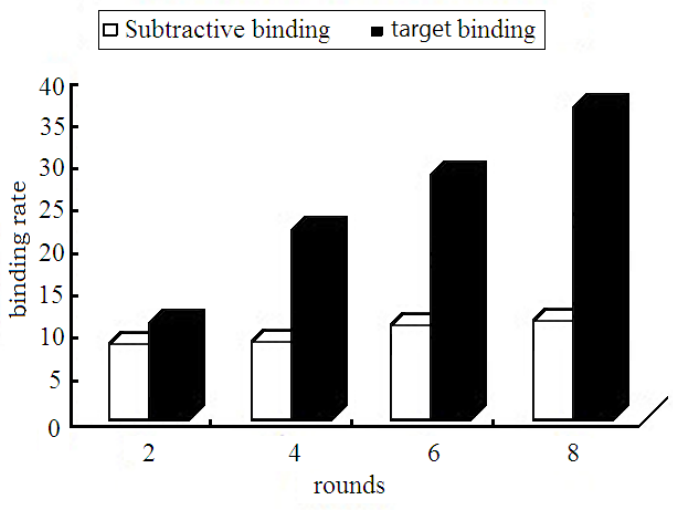

Figure 2. Aptamers dot blot binding to subtractive and target by radio-labeled aptamer binding assay. The intensity of radioactivity bound to target dot blot (black) steadily increases in every 2 rounds of selection.

Selection of positive clones

The ssDNA aptamer pool isolated after 8 rounds was transformed into *E. coli* JM109 and obtained as 49 bacterial clones. The length of DNA inserted into the vector was only 85 bp, which could not completely inhibit the activity of lacZ, so the β-galactosidase may only partially function. Light-blue bacterial colonies appeared in the blue-white selection, signifying that the selection efficiency might have been compromised. To ensure the accurate display of the insert direction, interlace PCR with consensus and specific primers was used to select positive clones. The electrophoresis band of the positive clones appeared at 154 bp. After PAGE, 40 positive clones (including 25 forward direction insertions and 15 inversion insertions), at a transformation rate of 81.6%, were obtained (Figure 3). Through further experiments by dot blot, we found that 19 aptamers were able to bind mouse IgG, and 2 aptamers had better affinities than the others. These 2 aptamers were further sequenced (Table 1) by Sangon Biotech Co. Ltd.

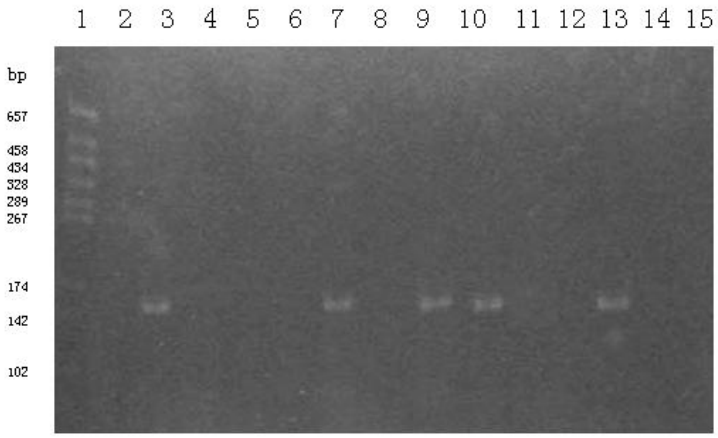

Figure 3. PAGE of the transformed clones by PCR with the combination of consensus and specific primers. *Lane 1* = molecular marker; *lanes 2* and *3* = positive control; *lanes 4* and *5* = negative control; *lanes 6-13* = samples; *lanes 14* and *15* = background control; 2, 4, 6, 8, 10, 12, 14 = DNA band by PCR with T7+P1; 3, 5, 7, 9, 11, 13, 15 = DNA band by PCR with T7+P2; 4 and 5, 6 and 7, 8 and 9, 10 and 11, 12 and 13 are the same samples.

Table 1. Sequences of the two selected aptamers.

Samples	Sequences
6	TAATACGACTCACTATAGCAATGGTACGGTACTTCCCCACTCA
	CCGGGTACCTGCCGCTCCCAAAAGTGCACGCTACTTTGCTAA
12	TAATACGACTCACTATAGCAATGGTACGGTACTTCCAAGCTAA
	CCCTCATCTGCGCGCTCCCAAAAGTGCACGCTACTTTGCTAA

Secondary structure assay

Analysis of the secondary structure of the DNA aptamers was performed using a free-energy minimization algorithm (http://rna.urmc.rochester.edu/RNAstructure.html). The secondary structures of the aptamers were characterized using the RNA Structure program (Version

5.3). All aptamers had a predicted secondary structure with 2 similar stem-loop structures. The secondary structures of the No. 6 and No. 12 DNA aptamers (Figure 4) suggest that the 2 stem-loop structures may play an important role in the binding mechanism of the selected aptamers.

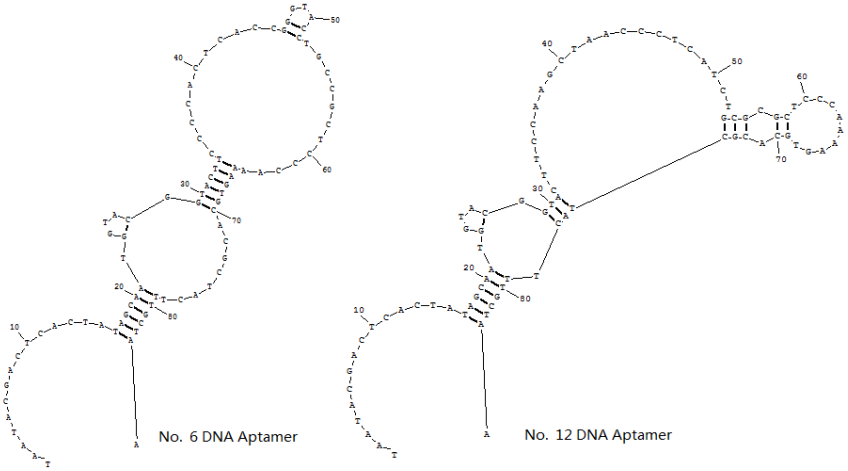

Figure 4. Structure analysis of DNA aptamers. The energetic favorable secondary structures of aptamer numbers 6 and 12, as determined using the RNA Structure program (Version 5.3).

Evaluation of binding capacity of the aptamers by dot blot

The blots of the mouse anti-HBs IgG, trypsin, solcoseryl albumin, and human serum that were incubated with [α-^{32}P]-labeled aptamers from different rounds of selection were further analyzed using the 2-D Gel Image Analysis Software Package (Melanie 3). Although an equimolecular amount of each protein was immobilized on the same membrane, the results showed that the aptamers bound exclusively with the mouse anti-HBs IgG (Figure 5A), demonstrating the DNA aptamer's specificity and affinity against the mouse anti-HBs IgG.

The dot blot results from mouse IgG Fc, IgG1, and IgG2a showed that, except for the mouse anti-HBs IgG, the aptamers were also able to universally bind with these IgG species, which were alternatively used as selection targets. Different levels of binding affinity by the aptamers toward the different IgG subclasses and the versatility of the selected aptamers to the subclasses of mouse IgG was also clearly demonstrated (Figure 5B). The commonly borne Fc fragment in the different mouse IgG subclasses accounted for this expected binding property.

EMSA assay of the aptamers

EMSA analysis was used to evaluate the binding affinity between the aptamers and mouse IgG. We used the aptamer of sample 6 to perform this experiment. The radioautography of the EMSA gel displayed binding bands of the aptamers with the target (Figure 6A). The bands could be clearly seen in lanes 1-4, which were loaded with the mixture of the mouse anti-HBs IgG (5, 10, 15, and 20 µg) and 70 ng [^{32}P]-labeled aptamers. As controls, the 5th lane was loaded with 70 ng [^{32}P]-labeled aptamers without mouse IgG, and the 6th lane was loaded with 20 µg mouse IgG without the [^{32}P]-labeled aptamer, and no bands developed in either

of these lanes. Thereafter, the gel was dyed by Coomassie brilliant blue and protein bands obtained are shown in Figure 6B. In lanes 1-4, the color density of the electrophoretic bands increased with increasing IgG concentration. These results conclusively demonstrated that the mouse IgG bound to the aptamers with high specificity and affinity.

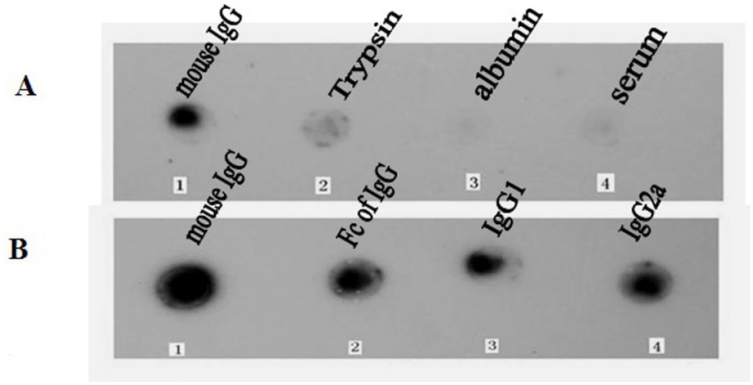

Figure 5. A. Autoradiography results of dot blot assay after aptamer incubation with **1.** mouse anti-HBV IgG (6 μg); **2.** trypsin (6 μg); **3.** solcoseryl albumin (6 μg); **4.** human serum (0.6 μL). **B.** Autoradiography results of dot blot assay after aptamer incubation with different mouse IgG subtypes. **1.** Mouse anti-HBs IgG (6 μg); **2.** IgG Fc (6 μg); **3.** IgG1 (6 μg); **4.** IgG2a (6 μg).

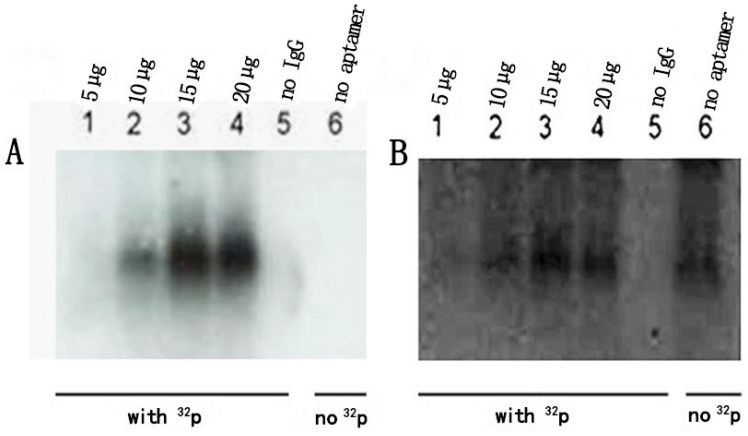

Figure 6. Electrophoretic mobility shift assay (EMSA) for aptamers. **A.** EMSA radioautography image of the binding complex of aptamers and IgG. *Lanes 1-4* = mixture of IgG (5, 10, 15, and 20 μg) and [^{32}P]-aptamers (70 ng); *lane 5* = [^{32}P]-aptamers (70 ng) without IgG, *lane 6* = IgG (20 μg) without [^{32}P]-aptamers. **B.** EMSA photograph after stained by Coomassie brilliant blue.

DISCUSSION

IgG is the most abundant immunoglobulin in humans. Affinity agents, such as anti-IgG antibodies, play an important role in immunology research, immune reaction-based bioanaly-

sis, and antibody purification. Antibodies are produced in biological systems by inducing an immune response to an antigen, but the immune response may fail when the target molecule, such as a protein, has structural similarity to an endogenous protein or when the antigen contains toxic compounds. In contrast, aptamers can be isolated by *in vitro* methods that are not affected by any target-induced biological response, thereby avoiding the limitations of antibody production. Therefore, aptamers serving as an IgG-recognizing agent have been the focus of great attention. Moreover, aptamers capable of recognizing the conservative Fc region, or those that can universally bind to different subclasses of the mouse IgG family, would be useful for more general applications. One drawback is that IgG consists of several subclasses and part of its structure is altered when it adapts to the various structures of antigens. In the conventional SELEX method, a single target is used to select the aptamer. The aptamer is intensively selected from an interaction with one IgG subclass species and will specifically bind with that IgG molecule at any possible site with high affinity, but will bind to other subclasses with poor affinity.

We aimed to obtain aptamers that could universally recognize a general range of IgG members, and therefore used a target replacement strategy to modify the standard SELEX procedure. In this new approach, multiple IgG subclass targets were sequentially used and replaced by another one SELEX. After the use of a different IgG subclass target in the next round of selection, only the aptamer candidates capable of binding to a site common between the 2 IgG subclasses were able to pass on to the next round of selection. Therefore, consecutive replacement of the 4 IgG subclasses should ultimately generate universal aptamers that could bind to all IgG subclasses. In this study, anti-HBs IgG, mouse IgG Fc, IgG1, and IgG2a were used to test the feasibility of this new approach.

During the screening process of SELEX, the preparation and purification of ssDNA is a necessary step. In this assay, we used the asymmetric PCR method to prepare ssDNA. The primer (with the same concentration) amplification products were used to prepare dsDNA, and then the dsDNA was used as the template to prepare ssDNA through asymmetric PCR (Figure 7). During the process of asymmetric PCR, the primers P1 and P2 were continuously consumed, and dsDNA continuously increased. When the restriction primer P2 (the low-concentration primer) was completely consumed, the PCR primed by the non-restriction primer P1 (the high-concentration primer) produced ssDNA. The oligonucleotide ssDNA prepared through asymmetric PCR had a higher concentration without nonspecific product bands, and was suitable for gel cutting and recovery, thereby providing a high-quality second library of oligonucleotide ssDNA for SELEX screening.

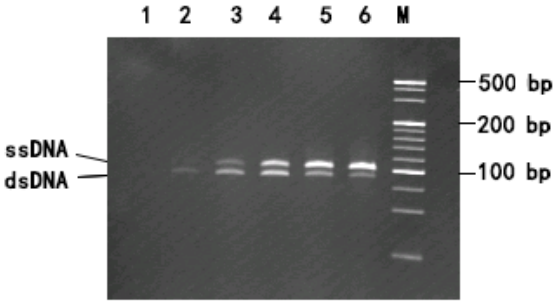

Figure 7. Method of asymmetric PCR to prepare ssDNA. *Lane M* = 20-bp DNA ladder marker; *lanes 1-6* = products respectively by the PCR cycles of 5, 10, 15, 20, 25, and 30.

Gel retardation, also called EMSA, is a special gel electrophoresis technique that is mainly used for *in vitro* studies of the interaction between DNA and specific proteins. Generally, the radiolabeled DNA fragments are bound to the purified protein, and the products are analyzed on the native gel. Compared to the free DNA, the protein-DNA compound has a lower mobility. Hence, the phenomenon of "retardation" will be observed on the electrophoretic bands of the protein-DNA compound, corresponding to the free DNA. This assay uses 0.5% agarose gel as the separation medium, and applies the gel retardation technique to verify the binding of the screened ligands and IgG for a clear result.

When foreign DNA is inserted into cloning vectors and transformed into bacteria, sequencing or restriction methods are necessary in order to identify positive clones. These methods are effective but are expensive or difficult. This assay uses a common primer of vectors and a specific primer of target fragments to increase and recombine the solution of bacteria directly through PCR, thus identifying the positive clones and the inserting direction of foreign DNA. This method may quickly and accurately identify the result of transformation of the recombined oligonucleotide dsDNA and vector PUC19 into *E. coli*, and to a certain extent, avoid the false positivity resulting from the contamination of target fragments.

Dot blot is a common nucleic acid and protein analysis method in biology and medicine, which may be used for semi-quantitative analyses. In this assay, the Melanie 3 software is used to implement the absorption spectrum of dot blot results against the obtained second library of oligonucleotides bound with IgG in order to select the oligonucleotide ligands that are best bound to IgG. This method is efficient, easy, quick, user-friendly, highly sensitive, and of low cost.

Due to the design of this assay, molecules such as skimmed milk powder are introduced in the screening process of SELEX. To a certain extent, the binding of oligonucleotides or other molecules may be avoided through the subtractive SELEX and target replacement strategy; however, the specific binding test is still required in order to identify the binding specificity of the selected oligonucleotide ligands with IgG. Through the dot blot analysis method, which is easy and highly sensitive, this assay conducts specificity identification on the optional nucleic acid ligands, and ultimately determines the specificity-identified IgG-bound nucleic acid ligands.

Use of a new target replacement strategy in SELEX has been demonstrated to successfully obtain DNA aptamers that are capable of recognizing and binding with the Fc region of multiple IgG subclasses. This new approach provides a simple and straightforward method for selecting aptamers with universal IgG binding capabilities, which may prove to be more useful for general biomedical probing and analytical applications. Target replacement strategy also offers the potential for selecting aptamers that can bind common regions of target molecules other than antibodies.

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