Biochemical and Biophysical Research Communications 452 (2014) 484–489

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Sequence-constructive SELEX: A new strategy for screening DNA aptamer binding to Globo H

Chin-Yu Wang, Chung-Yi Wu, Ting-Chun Hung, Chi-Huey Wong, Chung-Hsuan Chen *

Genomics Research Center, Academia Sinica, 128 Academia Road, Sec. 2, Nankang, Taipei 11529, Taiwan

ARTICLE INFO

Article history:
Received 4 August 2014
Available online 23 August 2014

Keywords:
Globo H
SELEX
DNA aptamer

ABSTRACT

We proposed to use a novel stepwise sequence-constructive SELEX method to develop DNA aptamers that can recognize Globo H which is a tumor-associated carbohydrate antigen. A combinatorial synthetic library that consisted of DNA molecules with randomized regions of 15-bases was used as the starting library for the first SELEX procedure. The input DNA library for the second round of SELEX consisted of the extension of the 5′ and 3′-ends with 7-bases that were randomized from four selected aptamers. The third round of SELEX was performed following the same procedures as described for the second round of SELEX. The experimental results indicate that the binding affinity of DNA aptamers to Globo H was enhanced when using the sequence-constructive SELEX approach. The selectivity of the DNA aptamers for related disaccharides, mannose derivatives, and Globo H analogs demonstrated the ability of the DNA aptamers to discriminate the presence of various glycans with different structures.

1. Introduction

Variations of cellular glycosylation patterns are often a hallmark of disease and can be considered a universal feature of cancer cells [1,2]. Studies have shown that carbohydrates on the surface of the cells play an essential biological role in diverse biological phenomena, such as cellular adhesion, molecular recognition [3], inflammatory response [4] and molecular changes that occur on malignant transformation [5]. Understanding the specificity of the interaction of cell-surface carbohydrates with antibodies and the ability to discriminate the presence of different glycans with relatively subtle structural differences are important for the development of new therapeutics and diagnostics.

Aptamers are functional DNA or RNA molecules that fold into well-defined structures and bind to a wide range of molecular targets, such as small molecules [6,7], proteins [8,9], organelles [10], viruses [11], and entire cells [12], with high affinity and specificity. Compared to antibodies, aptamer has a number of advantages: it has no or low toxicity; animals are not needed for their generation; it is robust against reducing conditions and heat treatment; and it can be easily synthesized and designed. Furthermore, the potential for automation [13] can significantly accelerate anti-glycan aptamer technology to become a critical part of the rapidly growing field of proteomics.

Aptamers are usually produced by polymerase chain reaction (PCR) with a chemical synthesis that contains random regions of nucleotides flanked by fixed sequences that are complementary to the primers. Moreover, aptamers with specific sequences have been obtained by Systematic Evolution of Ligands by Exponential Enrichment (SELEX) which was first reported in 1990 [6,8]. Statistically, only one molecule of each sequence exists by applying an input DNA library that contains a randomized region of 25-base during SELEX since a typical library has $\sim 1 \times 10^{15}$ DNA molecules. In the past, most SELEX were performed with random DNA sequence section as 30–40 nucleotides. Thus, it is difficult to produce enough molecules to cover all of the possible random DNA sequence combinations using a synthetic commercial sample when the random sequence region is longer than 25 bases since a typical DNA library has $\sim 10^{15}$ molecules.

Carbohydrates consist of only hydroxyl groups, which are able to form noncovalent bonds with significant stability; therefore, the carbohydrate "antigens" lack both the charged interactions and aromatic ring structures to have strong nucleic acid interactions. Currently, there are less than ten reports in the literature that have screened DNA aptamers for binding to carbohydrates such as cellulose [14], sialyllactose [15], cellobiose [16], (1→3)-β-D-glucans [17] and chitin [18]. These aptamers recognize their targets with K_d values from 10^{-5} to 10^{-7} M. The cell-surface glycosphingolipid Globo H is a member of a family of antigenic carbohydrates that are expressed on the cellular surface in many cancer cell lines, especially breast cancer cells [19–22]. Globo H tumor

* Corresponding author.
E-mail address: winschen@gate.sinica.edu.tw (C.-H. Chen).

http://dx.doi.org/10.1016/j.bbrc.2014.08.086
0006-291X/

antigen and the related truncated sequences for profiling the antibody interaction were reported by Huang et al. [23] Another method for analyzing glycan arrays with high sensitivity [24] is with nanoparticle probes and a conventional flatbed scanner for detection.

To avoid the lack of all possible combinations of nucleotide sequences from a long oligonucleotide in a synthetic commercial DNA library, we used random regions of only 14- or 15-base lengths in a combinatory DNA library during three SELEX procedures to ensure that all possible sequences participated in the selection process. In this study, we proposed a sequence-constructive SELEX method that is based on using magnetic streptavidin-coated beads for selection to generate high affinity and specific DNA aptamers that recognize the cancer antigen Globo H. The binding affinity of the DNA aptamers to Globo H was enhanced by extending the randomized sequence 7-bases on the 3′- and 5′-ends of the previously selected sequence. Disaccharide lactose, mannose 4 and mannose 9 were selectively recognized with DNA aptamers due to their structural differences from Globo H. Therefore, we believe that this method can be used to screen high affinity and specific DNA aptamers against other biologically important oligosaccharides. Furthermore, an increase in throughput by automation can help anti-glycan aptamer technology become an integral part of the rapidly growing field of proteomics.

2. Materials and methods

2.1. Materials

Randomized DNA oligonucleotide libraries, primers, selected aptamers and modified aptamers were purchased from Genomics BioSci & Tech (Taiwan). In a 50-μl PCR reaction, 100 pmol forward primer A (5′-GTACG AATTC ACGAG GTTGCC) and reverse primer A (5′-TAGAG GATCC GACTC CATGCT) were used to amplify selected DNA molecules. Forward primer B (5′-ATAGG AGTCG ACCGA CACGA A) and reverse primer B (5′-ATGAG CTTAG ATGTA GACGC ACATA) were only used for PCR amplification of "modified" aptamers in the determination of the structure of the aptamers. More details on "modified" aptamers will be given in the later section. Selected single-stranded DNA was amplified with 10 cycles of PCR (95 °C, 20 s; 60 °C, 30 s; 74 °C, 10 s) using Exsel DNA polymerase (Bertec Enterprise Co. Ltd., Taiwan). Fig. S1 in Supplementary data illustrates the schematic of the SELEX procedure used in this study, which was based on using streptavidin-coated magnetic beads for selection.

2.2. Constructive DNA pool and modified-DNA aptamers

Sequence-constructive SELEX strategy used to develop DNA aptamers that recognize Globo H with high affinity and specificity is shown in Fig. 1. Seven rounds of *in vitro* selection were included in each step of SELEX. For the first SELEX procedure, synthetic 57-mer oligonucleotides with a random region of 15-bases (5′-GTACG AATTC ACGAG GTTGCC $\mathbf{N_{15}}$ AGCAT GGAGT CGGAT CCTCTA) were used for seven *in vitro* selections. After cloning and sequencing, 37 different oligonucleotides were obtained to assay the binding affinity to Globo H, and four specific aptamers (No. 24, 28, 43, 55) were selected for the second round of SELEX.

By extending the four specific aptamers that were selected from the first round of SELEX with seven randomized bases from the 5′- and 3′-ends (GTACG AATTC ACGAG GTTGCC $\mathbf{N_7}$ XXXXX XXXXX XXXXX $\mathbf{N_7}$ AGCAT GGAGT CGGAT CCTCTA), where XXXXX XXXXX XXXXX was GACGT GCCGG TGTTG (No. 24); ATCGT CACGG CCATG (No. 28); GGTGT GGTGT CGCGT (No. 43) and AGGCA GCGTG GCCGT (No. 55), four randomized 14-base DNA libraries were separately generated to complete seven *in vitro* selections in the second round of SELEX.

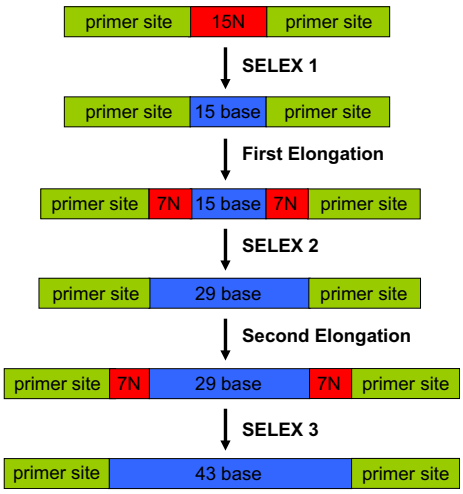

Fig. 1. The sequence-constructive SELEX method.

In total, 173 different sequences were obtained after the second round of SELEX from four randomized 14-base DNA libraries, and these sequences were used to assay the binding affinity with Globo H. The sequence with the highest affinity to Globo H was DNA aptamer No. 2411 (**TCTCAGG** GACGT GCCGG TGTTG **TCGCTAT**), which was selected to extend the length of the aptamer by seven bases at the 5′- and 3′-ends (5′-GTACG AATTC ACGAG GTTGCC $\mathbf{N_7}$ **TCTCAGG** GACGT GCCGG TGTTG **TCGCTAT** $\mathbf{N_7}$ AGCAT GGAGT CGGAT CCTCTA-3′), and seven *in vitro* selections were performed in the third round of SELEX. After cloning, sequencing, PCR, APCR, and PAGE purification, 92 different sequences were obtained after the third round of SELEX to assay the binding affinity with Globo H. The sequences of the DNA aptamers from the third round of SELEX to bound Globo H best (241163) and some of their modified sequences and positions are indicated in Table 1.

The proposed secondary structures of aptamers were obtained using the software from http://www.bioinfo.rpi.edu/applications/hybrid/quikfold.php which represented the lowest energy conformations are shown in Fig. 2. DNA aptamer 2411 from the second round of SELEX was modified at position 43 (G → A), 44 (T → G) or 45 (C → A) to study the effect of a hairpin structure on binding to Globo H. DNA aptamer 241163 from the third round of SELEX was modified at positions 27 and 28 (GG → TT) to transform the smaller hairpin structure into a linear structure from positions 20 to 28. The primer sequences were replaced by primer set B in DNA aptamer 241163 to investigate the change in binding affinity between DNA and Globo H due to the presence of two small hairpin structures near the terminal end of the DNA.

2.3. Binding assay of the isolated aptamers

Each individual aptamer was assayed for Globo H binding to obtain a preliminary binding affinity value. Commercial synthetic DNAs were used to confirm the binding affinities of the aptamers through binding assay experiments that were performed a minimum of three times. After the elution of aptamers at 95 °C from Globo H, the solutions were concentrated under a vacuum at low temperatures until they were dried. The dried aptamer was dissolved in 40 μL of pure water, and PCR was performed. Five microliters of the PCR product was analyzed by 1.6% agarose gel that was stained using SYBR Green II.

Table 1
The sequences of Globo H-binding DNA aptamers.

Aptamers	No. of SELEX	Sequences
24	**1**	GTACGAATTCACGAGGTTGCCGACGTGCCGGTGTTGAGCATGGAGTCGGATCCTCTA
28	**1**	GTACGAATTCACGAGGTTGCCATCGTCACGGCCATGAGCATGGAGTCGGATCCTCTA
43	**1**	GTACGAATTCACGAGGTTGCCGGTGTGGTGTCGCGTAGCATGGAGTCGGATCCTCTA
55	**1**	GTACGAATTCACGAGGTTGCCAGGCAGCGTGGCCGTAGCATGGAGTCGGATCCTCTA
2411	**2**	GTACGAATTCACGAGGTTGCC**TCTCAGG**GACGTGCCGGTGTTG**TCGCTAT**AGCATGGAGTCGGATCC TCTA
43A2411	**modified**	GTACGAATTCACGAGGTTGCC**TCTCAGG**GACGTGCCGGTGTT**A**TCGCTATAGCATGGAGTCGGATCC TCTA
44G2411	**modified**	GTACGAATTCACGAGGTTGCC**TCTCAGG**GACGTGCCGGTGTTG**G**CGCTATAGCATGGAGTCGGATCC TCTA
45A2411	**modified**	GTACGAATTCACGAGGTTGCC**TCTCAGG**GACGTGCCGGTGTTGT**A**GCTATAGCATGGAGTCGGATCC TCTA
24113	**3**	GTACGAATTCACGAGGTTGCC*AAAATAC***TCTCAGG**GACGTGCCGGTGTTG**TCGCTAT***TGTACTG***AGCA TGGAGTCGGATCCTCTA
p241163	**3**	ATAGGAGTCGACCGACACGAA*ACTCTGG***TCTCAGG**GACGTGCCGGTGTTG**TCGCTAT***CCAATGC***TATG TGCGTCTACATCTAAGCTCAT
241163	**3**	GTACGAATTCACGAGGTTGCC*ACTCTGG***TCTCAGG**GACGTGCCGGTGTTG**TCGCTAT***CCAATGC***AGCA TGGAGTCGGATCCTCTA
27T28T241163	**modified**	GTACGAATTCACGAGGTTGCC*ACTCT**TT***TCTCAGG**GACGTGCCGGTGTTG**TCGCTAT***CCAATGC***AGCA TGGAGTCGGATCCTCTA

The DNA sequence from 5′- to 3′-end, primer sequences on two fixed extremities.
The sequences from the first SELEX are underlined.
The sequences from the second SELEX are boldface.
The sequences from the third SELEX are italic.
The modified oligonucleotides are highlight red letters.
The primer B set sequence is in two square circles.

2.4. Selectivity assay of DNA aptamer 241163 for six different carbohydrates

We selected six biotin-labeled carbohydrates (Globo H, lactose, Man 4, Man 9, Gb4 and Gb5, Fig. S2 in Supplementary data) to determine the specificity of the DNA aptamers. The procedure used for the biotin-labeled carbohydrates that were conjugated to streptavidin-coated magnetic beads was the same as mentioned in the SELEX procedures. The ratio of biotinylated sugar to streptavidin-coated magnetic beads was 10.

2.5. Determination of dissociation constant (K_d) of aptamer

AutoITC200 (isothermal titration calorimetry, Microcal, GE Healthcare) was used to measure K_d of DNA aptamer binding to Globo H. The reaction ratio of aptamer with Globo H was 1:3.

3. Results and discussion

3.1. In vitro selection of Globo H binding DNA aptamers

According to calculations, there are approximately one million molecules for a randomized 15-base nucleotide sequence between two fixed primer sites in a synthetic commercial DNA library sample which contains 10^{15} DNA molecules in total ($10^{15}/4^{15} = \sim 10^6$). In order to determine this approach to be able to pick up the aptamer with strong interaction with the target under a tremendous number of DNAs with different sequences, we used a DNA sample to estimate PCR efficiency. 10^7 DNA aptamers with specific sequence which is complementary with biotinylated DNA on streptavidin-coated beads was tested in a simulated SELEX process along with 10^{14} nonspecific DNA molecules in the sample. We found that a PCR band of specific DNA appeared after seven rounds of selection.

Based on the synthetic limitations of DNA and the simple statistical calculations, there is approximately one molecule of each sequence in a 10^{15} synthetic commercial DNA pool that contains a randomized 25-base sequence ($4^{25} = \sim 10^{15}$). Most sequence combinations will not be represented if the DNA library with a variable region is significantly longer than 25 bases. For example, the possibility is 1 in 10^9 for the aptamer with the best sequence to couple with the target to exist in the pool when the length of the variable region is 40 bases. The schematic streptavidin-coated magnetic bead SELEX method is shown in Fig. 1. Before our sequence-constructive SELEX method, we did traditional SELEX with fifteen selection rounds by using synthetic 60mer randomized sequence DNA library (total 102mer in length) for binding Globo H. The selection results showed the dissociation constant of 60mer randomized sequence DNA for Globo H was obtained as ~6 μM.

In this study, we used a randomized sequence that was 15 bases in length in the region of our combinatory DNA library in the first SELEX process to assure that every possible sequence participated in the selection process. According to statistical calculation, about three million molecules of each sequence should exist for reaction before the next two SELEX processes which are to extend seven nucleotides at both 3′ and 5′ ends from the input DNA library. Seven rounds of selection were performed for each SELEX procedure, and the sequence-constructive SELEX strategy is shown in Fig. 1.

After the first round of SELEX, four specific aptamers (24, 28, 43, and 55; sequences shown in Table 1) were selected from 37 colonies which are represented with 37 different sequences. After the second SELEX procedure was performed, DNA aptamer 2411 was selected as the next input DNA, and the same method to extend the oligonucleotide was performed. Twenty-one *in vitro* selections were pursued during the three rounds of SELEX to generate high affinity and specificity for DNA aptamers to couple with Globo H.

The lengths of the DNA aptamers obtained from the first, second, and third SELEX processes were 57, 71 and 85-mers,

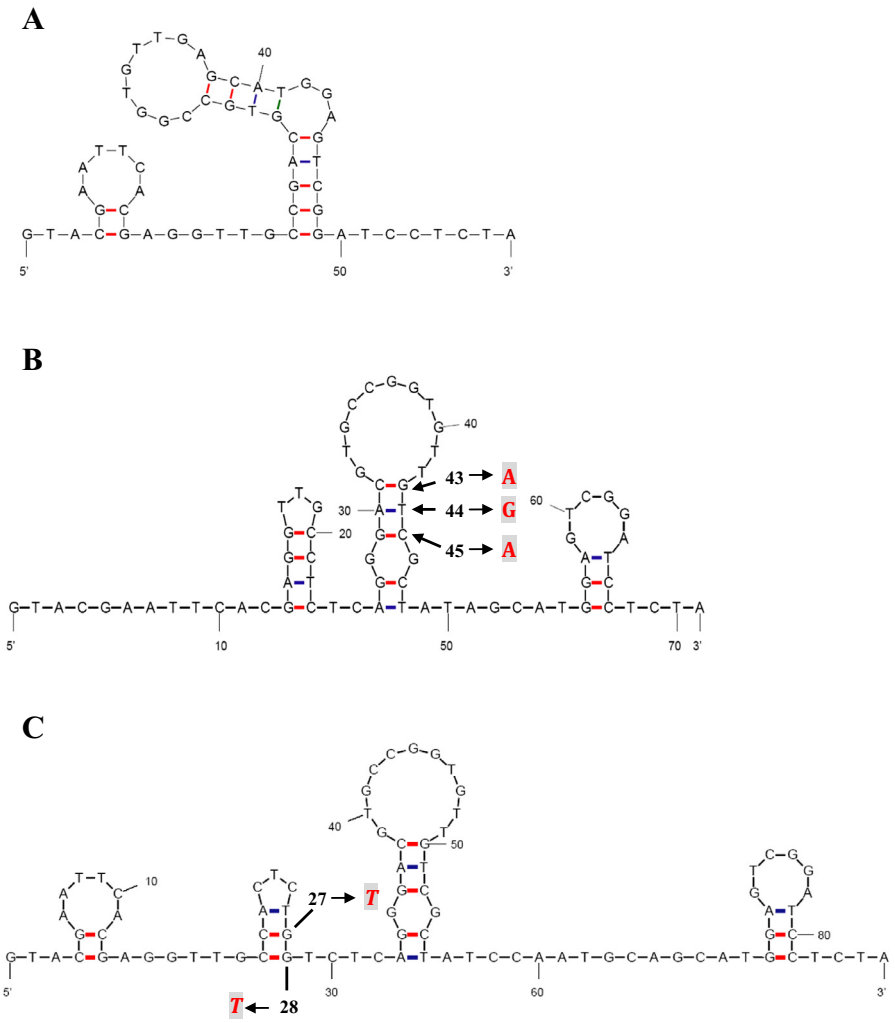

Fig. 2. Proposed secondary structures of selected aptamers. (A) 24; (B) 2411; (C) 241163.

respectively. Ten cycles of PCR were performed to assay the binding affinities of Globo H to the three different DNA aptamers which were obtained from the three SELEX steps and determined by gel electrophoresis. Increased binding affinities were observed as the length of the DNA aptamers was increased. The binding affinity was primarily determined by the interaction between Globo H and the randomized DNA sequence. The background of non-specific streptavidin bounding to biotin was also subtracted for distinguishing the binding ability of DNA to Globo H. As shown in Fig. 3I, nonspecific binding did not increase as the DNA aptamer length increased. According to the K_d values of aptamer 24, 2411 and 241163 determined by isothermal titration calorimetry, the K_d of aptamer 241163 (0.7 μM) from the third round of SELEX was thirty times better than aptamer 24 (23 μM) result from the first SELEX round as displayed in Fig. S3 in Supplementary data. Both electrophoresis and dissociation constant results have demonstrated further increase in binding affinity was obtained by applying only 14 or 15 base lengths of a random region to the combinatory DNA library during three SELEX procedures.

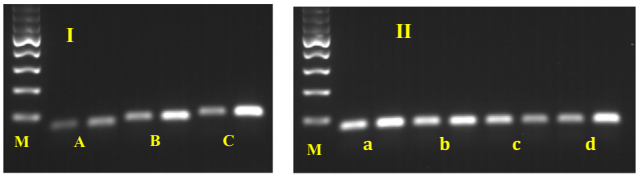

Fig. 3. I: Assay of the binding affinities of DNA aptamers of three different lengths from three continuous SELEX processes to Globo H. (A) DNA aptamer 24 (57-mer) in the presence of biotin only (left) or Globo H (right) on streptavidin magnetic beads. (B) DNA aptamer 2411 (71-mer) in the presence of biotin only (left) or Globo H (right) on streptavidin magnetic beads. (C) DNA aptamer 241163 (85-mer) in the presence of biotin only (left) or Globo H (right) on streptavidin magnetic beads. II: Assay of the binding affinity between modified-DNA aptamer 2411 and Globo H. (a) 43A2411 (position 43 G → A) in the presence of biotin only (left) or Globo H (right) on streptavidin magnetic beads. (b) 44G2411 (position 44 T → G) in the presence of biotin only (left) or Globo H (right) on streptavidin magnetic beads. (c) 45A2411 (position 45 C → A) in the presence of biotin only (left) or Globo H (right) on streptavidin magnetic beads. (d) DNA aptamer 2411 in the presence of biotin only (left) or Globo H (right) on streptavidin magnetic beads. M: 100-bp marker.

3.2. Binding assay of the modified-DNA aptamers with Globo H

Although the proposed secondary structure of aptamer 24 is not related to aptamer 2411 and 241163, the sequence is a part of aptamers 2411 and 241163 (Fig. 2). To explore the effects of the sequence and the secondary structure of the selected DNA aptamers on the binding affinity between DNA and Globo H, we modified three nucleotides in the hairpin stem region of aptamer 2411. The modified nucleotide positions are indicated in Fig. 2. Fig. 3II shows the gel electrophoresis results of the modified 2411 DNA aptamer obtained from the second round of SELEX. The effect of binding affinity of Globo H to modified-DNA aptamer

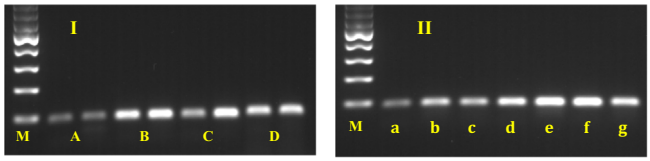

Fig. 4. I: Comparison of the binding affinity of DNA aptamer 24113, modified 241163 (27T28T241163, position 27 and 28 GG → *TT*), aptamer 241163, and aptamer p241163 using primer set B to Globo H. (A) DNA aptamer 24113 in the presence of biotin only (left) or Globo H (right) on streptavidin magnetic beads. (B) 27T28T241163 in the presence of biotin only (left) or Globo H (right) on streptavidin magnetic beads. (C) DNA aptamer 241163 in the presence of biotin only (left) or Globo H (right) on streptavidin magnetic beads. (D) p241163 in the presence of biotin only (left) or Globo H (right) on streptavidin magnetic beads. II. Selectivity assay of DNA aptamer 241163 to six different sugars. The PCR products from the binding assay of DNA aptamer 241163 in the presence of (a) biotin only, (b) lactose, (c) mannose 4, (d) Gb4, (e) Gb5, (f) Globo H, and (g) mannose 9 conjugated to streptavidin-coated magnetic beads. M: 100-bp DNA marker.

2411 can be clearly observed. Specifically, only the central hairpin structure of aptamer 2411 was eliminated by modifying one nucleotide at the stem site, and the resulting binding affinity between DNA and Globo H was altered. Modification to the nucleotide sequence at position 45 in DNA aptamer 2411 was more important than modification at the other two positions (43: Fig. 3IIa and 44: Fig. 3IIb) because the binding affinity was significantly decreased between DNA aptamer 45A2411 and Globo H, which was caused by exchanging a C into an A at position 45 as shown in Fig. 3IIc. In addition, we observed an increase in background binding for all three modified aptamers.

The structural effect of the small hairpin can be studied with DNA aptamer 24113 from the third SELEX process (Table 1), which possesses a structure similar to 241163; however, there were no small hairpin structures between positions 20 and 28. A loss of binding affinity for DNA aptamer 24113 to Globo H was observed (compare Fig. 4IA with 4IC) and exhibited no discrimination from biotin. To study the effect of the smaller hairpin structure in DNA aptamer 241163 between two hairpin structures at the 5′-end, we modified GG at positions 27 and 28 to TT to change the hairpin into a linear structure (27T28T241163). Although the binding affinity is not apparently changed, the background binding is significantly increased by using modified aptamer 27T28T241163 (Fig. 4IB). These results demonstrate that the binding affinity between the aptamers and Globo H was influenced by both the sequence and structure of the oligonucleotides.

3.3. Binding assay with Globo H using a different primer set with DNA aptamer 241163

In addition to the two hairpin located in the center of DNA aptamer 241163, there were two small hairpins formed at the terminal site due to the primer sequences. To determine the role of the two hairpins in aptamer 241163 on the binding affinity to Globo H, we used primer B set to assay their effect. The primer sequences of DNA aptamer 241163 were replaced to obtain DNA p241163. No distinguishable binding affinity was obtained between biotin and Globo H with p241163 DNA (Fig. 4ID), which indicates that the primer sequences can provide some contribution to the binding affinity to Globo H. The binding affinity for Globo H of DNA aptamer 241163 from the third SELEX selection was the best by using primer A set is shown in Fig. 4IC.

3.4. Selectivity of DNA aptamer 241163 to six different carbohydrates

We used lactose, Man4, Man9, Gb4, Gb5 and Globo H to determine the specificity of selected DNA aptamers to Globo H. (Fig. S2 in Supplementary data). The binding of DNA aptamer 241163 was the greatest for Globo H (Fig. 4IIf), which had a similar binding affinity with Gb5, and was reduced for Gb4, Man9, lactose, and Man4 (Fig. 4II). The binding specificity of DNA aptamer 241163 was greater for Gb4 than for Man4. Branch-type mannose 9 and Gb4 had a similar binding affinity for Globo H. As shown in Fig. S2, the length and type of sugars were the major factors that DNA aptamer 241163 selectively recognized for these carbohydrates. Disaccharide lactose and mannose 4 have the least affinity with DNA aptamer 241163 because of their significant structural differences from Globo H.

In conclusions, we report a DNA aptamer which can recognize Globo H with the proposed stepwise sequence-constructive SELEX process that used streptavidin-coated magnetic beads to produce DNA aptamers for Globo H with high affinity and specificity. By applying only 14 or 15 base lengths of a random region to the combinatory DNA library during three SELEX procedures, we were able to ensure that every possible sequence existed in the randomized DNA library to participate in the selection process. The binding ability of DNA aptamers to Globo H was enhanced when the lengths of the randomized regions were extended from the 5′ and 3′ ends by seven nucleotides after the first SELEX process. A further increase in binding affinity was obtained after elongation of the DNA aptamers. Moreover, we found that the structure of the aptamers after elongation could significantly change the binding affinity between DNA and Globo H with only one nucleotide change. The hairpin structures from the selected primer sequences also played an important role in the binding affinity between DNA and Globo H. The length and type of carbohydrates were the major factors that affected the selectivity of the DNA aptamers to these saccharides. The development of the high affinity ligands to recognize a variety of small motifs in different oligosaccharides is expected to be a valuable diagnostic tool.

Acknowledgments

We would like to acknowledge the financial support by the Genomics Research Center, Academia Sinica; the National Science Council (Grant No. NSC 99-2113-M-001-002-MY3) and the National Health Research Institutes (Grant No. NHRI-EX101-9803EI) in Taiwan.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.08.086.

References

[1] E. Meezan, H.C. Wu, P.H. Black, P.W. Robbins, Comparative studies on the carbohydrate-containing membrane components of normal and virus-transformed mouse fibroblasts. II. Separation of glycoproteins and glycopeptides by sephadex chromatography, Biochemistry 8 (1969) 2518–2524.
[2] D.H. Dube, C.R. Bertozzi, Glycans in cancer and inflammation – potential for therapeutics and diagnostics, Nat. Rev. Drug Discov. 4 (2005) 477–488.
[3] H. Lis, N. Sharon, Lectins: carbohydrate-specific proteins that mediate cellular recognition, Chem. Rev. 98 (1998) 637–674.
[4] G.A. Turner, N-glycosylation of serum proteins in disease and its investigation using lectins, Clin. Chim. Acta 208 (1992) 149–171.
[5] G. Pancino, E. Osinaga, C. Charpin, D. Mistro, J.P. Barque, A. Roseto, Purification and characterisation of a breast-cancer-associated glycoprotein not expressed in normal breast and identified by monoclonal antibody 83D4, Br. J. Cancer 63 (1991) 390–398.
[6] A.D. Ellington, J.W. Szostak, *In vitro* selection of RNA molecules that bind specific ligands, Nature 346 (1990) 818–822.
[7] E. Vianini, M. Palumbo, B. Gatto, In vitro selection of DNA aptamers that bind L-tyrosinamide, Bioorg. Med. Chem. 9 (2001) 2543–2548.
[8] C. Tuerk, L. Gold, Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase, Science 249 (1990) 505–510.

[9] S. Ringquist, T. Jones, E.E. Snyder, T. Gibson, I. Boni, L. Gold, High-affinity RNA ligands to *Escherichia coli* ribosomes and ribosomal protein S1: comparison of natural and unnatural binding sites, Biochemistry 34 (1995) 3640–3648.
[10] W.H. Pan, R.C. Carven, Q. Qiu, C.B. Wilson, J.W. Wills, S. Golovine, J. Wang, Isolation of virus-neutralizing RNAs from a large pool of random sequences, Proc. Natl. Acad. Sci. USA 92 (1995) 11509–11513.
[11] K.N. Morris, K.B. Jensen, C.M. Julin, M. Weil, L. Gold, High affinity ligands from in vitro selection: complex targets, Proc. Natl. Acad. Sci. USA 95 (1998) 2902–2907.
[12] J.C. Cox, A.D. Ellington, Automated selection of anti-protein aptamers, Bioorg. Med. Chem. 9 (2001) 2525–2531.
[13] D. Eulberg, K. Buchner, C. Maasch, S. Klussmann, Development of an automated in vitro selection protocol to obtain RNA-based aptamers: identification of a biostable substance P antagonist, Nucleic Acids Res. 33 (2005) e45.
[14] J.B. Benjami, R.B. Ronald, In vitro selection and characterization of cellulose-binding DNA aptamers, Nucleic Acids Res. 35 (2007) 6378–6388.
[15] M.M. Masud, M. Kuwahara, H. Ozaki, H. Sawai, Sialyllactose-binding modified DNA aptamer bearing additional functionality by SELEX, Bioorg. Med. Chem. 12 (2004) 1111–1120.
[16] Q. Yang, I.R. Goldstein, H.Y. Mei, D.R. Engelke, DNA ligands that bind tightly and selectively to cellobiose, Proc. Natl. Acad. Sci. USA 95 (1998) 5462–5467.
[17] S.Y. Low, J.E. Hill, J. Peccia, DNA aptamers bind specifically and selectively to (1 → 3)-β-d-glucans, Biochem. Biophys. Res. Commun. 378 (2009) 701–705.
[18] E. Fukusaki, T. Kato, H. Maeda, N. Kawazoe, Y. Ito, A. Okazawa, S. Kajiyama, A. Kobayashi, DNA aptamers that bind to chitin, Bioorg. Med. Chem. Lett. 10 (2000) 423–425.
[19] S. Zhang, C. Cordon-Cardo, H.S. Zhang, V.E. Reuter, S. Adluri, W.B. Hamilton, K.O. Lloyd, P.O. Livingston, Selection of tumor antigens as targets for immune attack using immunohistochemistry: I. Focus on gangliosides, Int. J. Cancer 73 (1997) 42–49.
[20] S. Hakomori, Y. Zhang, Glycosphingolipid antigens and cancer therapy, Chem. Biol. 4 (1997) 97–104.
[21] S. Mènard, E. Tagliabue, S. Canevari, G. Fossati, M.I. Colnaghi, Generation of monoclonal antibodies reacting with normal and cancer cells of human breast, Cancer Res. 43 (1983) 1295–1300.
[22] V. Kudryashov, G. Ragupathi, I.J. Kim, M.E. Breimer, S.J. Danishefsky, P.O. Livingston, K.O. Lloyd, Characterization of a mouse monoclonal IgG3 antibody to the tumor-associated globo H structure produced by immunization with a synthetic glycoconjugate, Glycoconj. J. 15 (1998) 243–249.
[23] C.Y. Huang, D.A. Thayer, A.Y. Chang, M.D. Best, J. Hoffmann, S. Head, C.H. Wong, Carbohydrate microarray for profiling the antibodies interacting with Globo H tumor antigen, Proc. Natl. Acad. Sci. USA 103 (2006) 15–20.
[24] C.H. Liang, C.C. Wang, Y.C. Lin, C.H. Chen, C.H. Wong, C.Y. Wu, Iron oxide/gold core/shell nanoparticles for ultrasensitive detection of carbohydrate–protein interactions, Anal. Chem. 81 (2009) 7750–7756.