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RESEARCH PAPER

Selection and truncation of aptamers for ultrasensitive detection of sulfamethazine using a fluorescent biosensor based on graphene oxide

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Abstract

We developed a fluorescent aptamer/graphene oxide (GO)–based biosensor to detect sulfamethazine (SMZ) residues in animal-derived foods. The SMZ-bound aptamers were identified and screened with an improved GO-SELEX technique using non-immobilizing ssDNA library. After seven rounds of selection, six SMZ aptamers were sequenced and analyzed for secondary structure, and their affinity and specificity were assessed by binding assays. The truncated aptamer (SMZ1S: 5′-CGTTAGACG-3′) with a unique stem-loop structure showed the highest affinity (K_d = 24.6 nM) to SMZ and was used to develop a GO-based fluorescent aptasensor. The binding mechanism between SMZ1S and SMZ was further analyzed by molecular docking. Under optimal conditions, the fluorescent aptasensor showed low detection limits (0.35 ng/mL) and a wide dynamic linear range (from 2 to 100 ng/mL). The aptasensor was also validated against real samples spiked with SMZ, which showed a fluorescence recovery from 93.9 to 108.8% and a coefficient of variation of < 12.7%. Taken together, these results suggest that this novel aptasensor can be used to sensitively, selectively, and accurately detect SMZ residues in foods.

Keywords ssDNA aptamer · Aptasensor · Sulfamethazine · Fluorescence · Graphene oxide · SELEX

Introduction

Sulfamethazine (SMZ) is a sulfonamide antibiotic that is often used to treat and prevent various protozoan and bacterial infections in livestock [1, 2]. However, the presence of SMZ residues in meat and other animal-derived foods can cause allergic or toxic reactions in humans and can interfere with intestinal flora by encouraging growth of resistant bacteria [3, 4]. From both veterinary and human use, a large part of SMZ may be excreted into the environment through urine, sweat, and feces without being metabolized, which is harmful to the ecological environment. As a result, the regulatory bodies of the European Union, the USA, and China have set maximum residue limit (MRL) of SMZ in animal-derived food products at 100 μg/kg [2, 5]. Currently, SMZ levels in food products are determined by high-performance liquid chromatography-ultraviolet [6, 7], liquid chromatography-tandem mass spectrometry [8–10], liquid chromatography [11], capillary electrophoresis-tandem mass spectrometry [8, 12], and immunoassays [13–16]. However, these methods are time-consuming and technically demanding, and require expensive equipment [17, 18]. There is an urgent need to develop a rapid and highly sensitive and accurate technique to detect SMZ in animal tissues and products in order to ensure food safety.

Aptamers, also known as chemical antibodies, are short single-stranded DNA or RNA that can fold into a complex three-dimensional structure and bind specifically to target molecules with high affinities [19, 20]. Aptamers are selected in vitro by a process known as systematic evolution of ligands by exponential enrichment (SELEX), such as multi-GO-SELEX, capture-SELEX, and capillary electrophoresis (CE)-SELEX [21]. Different target aptamers have been applied in biosensor platforms among various nano-materials such as gold nanoparticles [22], polymer nanobelt [23], and electrochemical [24–26]. They offer several advantages over traditional antibodies, including ease of production and modification, target diversity, low cost to prepare, high stability, high specificity, minimal toxicity, and multiple regeneration

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without loss of binding ability [4, 27]. Various assays have been developed in recent years using aptamers as recognition mechanisms for detection [28–30]. For example, magnetic bead–based SELEX is commonly used to select aptamers specific for small molecules, proteins, and cells; however, this method usually requires immobilizing targets on magnetic beads and multiple screening rounds [20, 21, 31, 32]. Therefore, it is necessary to develop rapid and more efficient methods for screening aptamers in order to improve aptamer performance and reduce screening costs.

Graphene oxide (GO) has unique electronic characteristics such as facile surface modification, good conductivity, large surface area, high fluorescence quenching efficiency, and good water dispersibility due to its abundant oxygen-containing functional groups, all of which makes it highly suitable as a biosensor [18]. In fact, GO-assisted non-immobilized SELEX (GO-SELEX) has been developed as an alternative to magnetic beads in efforts to improve the efficiency of aptamer screening [17, 33]. In addition, GO-SELEX is particularly suitable for screening aptamers against small molecule targets since the absence of covalent bindings preserves the structure of the target molecules (in comparison with magnetic bead–based SELEX) and simplifies the operation. Furthermore, GO is also suitable for developing biosensors based on fluorescence resonance energy transfer (FRET) because of its extensive cross-sectional area and electronic excitation energy transfer [18]. GO-based fluorescent sensor platforms have been developed based on the interactions between fluorophore-labeled aptamers and the GO surface—in the absence of target analytes—via electrostatic and π-π stacking interactions, which quenches the fluorescence by FRET; reversely, the dissociation of the aptamers from the GO surface—in the presence of the target analytes—restores the fluorescence intensity, which can be quantified. These GO-aptamer biosensors can distinguish multiple analytes based on different degrees of fluorescence quenching [17, 34]. This strategy may be more effective for target aptamers with short sequences, since short aptamers have weaker adsorption capacity to the GO surface than long aptamers and the aptamer-ligand bindings are more reliable [32, 35, 36].

Herein, we report for the selection of aptamers that bind SMZ with high affinity in vitro based on improved the GO-SELEX method, and optimized the binding affinity by truncating the sequence to the minimum binding domain. To the best of our knowledge, this is the first report of the shortest aptamer among sulfonamides. We then use molecular docking methods to study the mechanism of aptamer binding to SMZ, and the results showed that the molecular docking of the computational model was completely consistent with the experimental analysis. Furthermore, we developed a rapid, simple, selective, and sensitive GO-based fluorescent aptasensor for the detection of SMZ in animal-derived foods.

Materials and methods

Reagents

SMZ and other sulfonamide standards, and GO were purchased from Sigma-Aldrich (St. Louis, MO, USA). Streptavidin magnesphere® paramagnetic particles were purchased from Promega Corporation (Madison, WI, USA). All other chemicals, such as 2× SYBR Premix Ex Taq II, NaCl, KCl, $MgCl_2$, and KH_2PO_4, were obtained from Beijing Chemical Reagent Company (Beijing, China). Deionized water was purified using Millipore Milli-Q Ultrapure Water System (Bedford, MA) and was used for all experiments. All chemicals were of analytical grade and were used as received unless indicated otherwise.

DNA library and primers

The DNA library (N40 Lib), FAM-forward primer (FP), and biotin-reverse primer (RP) were synthesized by Sangon Biotech (Shanghai, China). The initial single-stranded DNA (ssDNA) library (5′-FAM-GACAGGCA GGACACCGTAAC-N40-CTGCTACCTCCCTC CTCTTC-3′, 80 mer) contained a random 40-nucleotide (nt) region flanked by a 20-nt primer-specific sequence (see Electronic Supplementary Material (ESM) Table S1). The library and primers were each diluted to 500 nM and 50 μM with binding buffer (20 mM Tris-HCl, 100 mM NaCl, 2 mM $MgCl_2$, 5 mM KCl, 1 mM $CaCl_2$, pH 7.4) and stored at − 20 °C until use.

In vitro selection of aptamers

GO-SELEX

The GO-SELEX process (positive selection) for SMZ aptamer screening is outlined in Fig. 1. Briefly, 200 μL N80 Lib (500 nM) was denatured at 95 °C for 10 min, rapidly cooled on ice for 10 min and incubated at room temperature in the dark for 25 min. For the initial round, 200 μL of 500 nM N80 Lib was incubated with 7 μL SMZ (4 μg/mL) for 1 h at 25 °C with constant shaking. The ssDNA/GO was then added at a mass ratio of 1:24, and the reaction mixture was incubated at room temperature with gentle shaking for 20 min, and centrifuged at 15,000 rpm for 10 min at 4 °C. The supernatant containing the SMZ-bound aptamers was collected, and the unbound ssDNA (i.e., ssDNA did not interact with the SMZ)

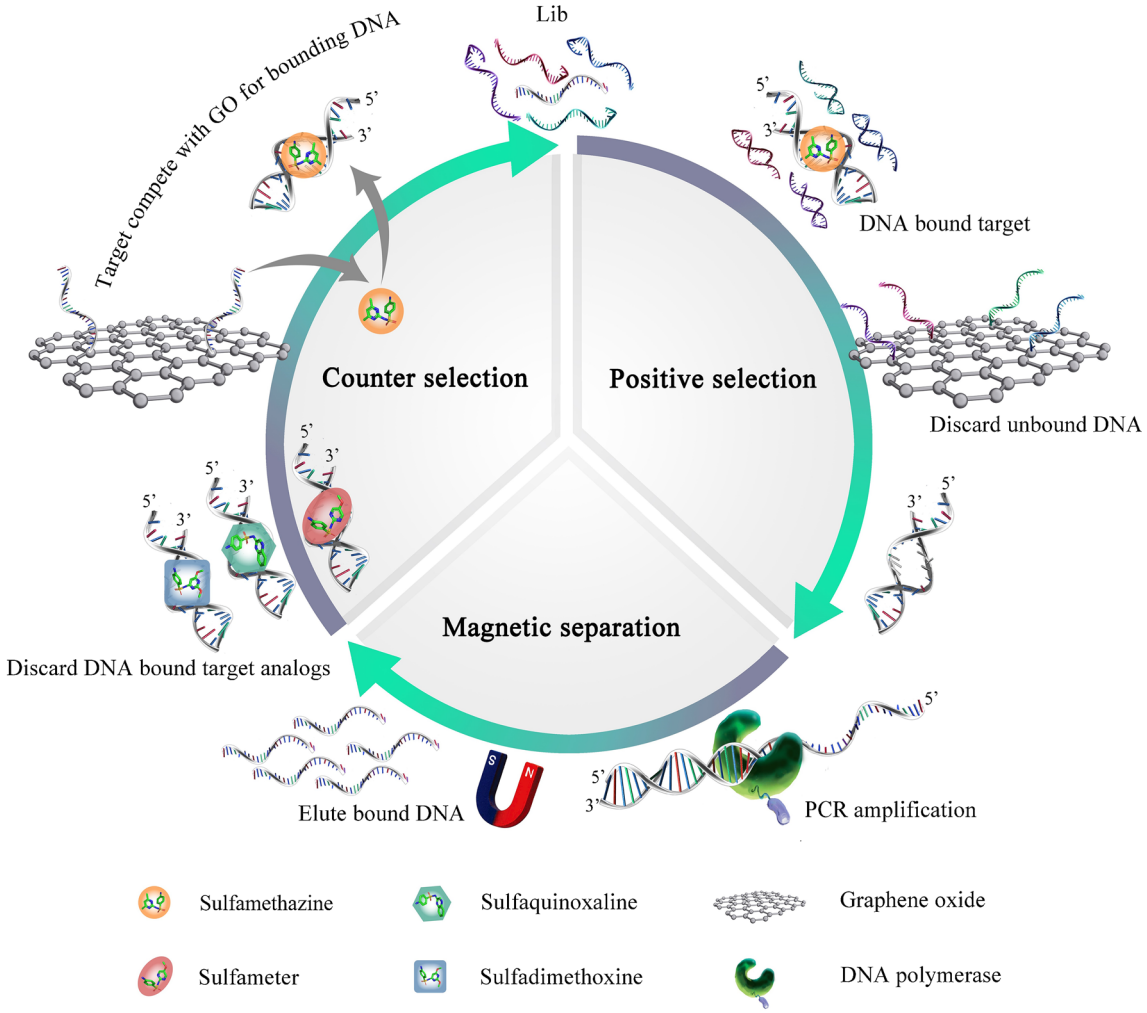

Fig. 1 Aptamer selection for SMZ. Schematic outline of the positive selection, magnetic separation and counter selection steps for screening high-affinity aptamers for SMZ

that physically adsorbed on the sediment was discarded. The collected aptamers in the supernatant were treated with 25 μL 3 M NaAc and 500 μL absolute ethanol for 20 min at − 20 °C and subsequently centrifuged at 15,000 rpm for 15 min at 4 °C. The supernatant was discarded and the precipitate was re-dissolved in 70% ice-cold ethanol, centrifuged again as indicated above, and the precipitate was re-dissolved in 60 μL deionized water. The fluorescence intensity of the ssDNA was measured using a Varioskan™ LUX Multimode Microplate Reader (Thermo Fisher Scientific Inc., Wilmington, USA), and its recovery rate was estimated as the percentage of bound ssDNA relative to the initially added ssDNA.

PCR amplification

The SMZ-bound ssDNA was amplified using the FAM-FP and Biotin-RP primers. The reaction mixture for the initial PCR consisted of 60 μL 2× Taq Master Mix, 0.8 μL FAM-FP (50 μM), 0.8 μL Biotin-RP (50 μM), and 60 μL template, and the cycling parameters were set as follows: 95 °C for 5 min, followed by 10 cycles at 95 °C for 30 s, 59 °C for 30 s and 72 °C for 30 s, and 72 °C for 5 min, and PCR products dilute to 600 μL. A second amplification was performed using 75 μL of the PCR products, 75 μL of 2× SYBR Premix Ex Taq II, and 1 μL of each primer with the same cycling conditions. The fluorescence intensity of SYBR-labeled ssDNA was measured at 520 nm (λ_{exc} = 470 nm) using a Varioskan ™ LUX Multimode Microplate Reader. The amplified products were electrophoresed in 15% (w/v) non-denaturing poly(acrylamide) gel at 130 V for 50 min to verify the presence of dsDNA. A final round of amplification was performed using 400 μL of PCR products, 400 μL of 2× Taq Master Mix, and 5.3 μL of each primer under the optimal conditions.

Preparation of secondary PCR library

The amplified dsDNA was incubated with 800 μL streptavidin magnesphere® paramagnetic particles in 0.1 mg/mL BSA in neutral PBS (pH 7.4) for 30 min at 25 °C. After washing thrice with PBS (pH 7.4), the supernatant was removed through a magnetic grate, and 50 μL 0.05 M NaOH was added. The mixture was agitated for 2 min to denature the dsDNA into ssDNA, followed by addition of 100 μL 2× Tri-HCl solution and 25 μL diethylpyrocarbonate-treated water (Sangon Biotech, China). After gentle shaking for 1 min, the solution was separated through a magnetic grate, and 25 μL 0.1 M HCl was added to the supernatant. The ssDNA library was isolated using a magnetic separator and quantified. To assure specificity of the candidate aptamers, counter-SELEX was performed using three structural analogs of the SMZ (i.e., sulfameter, sulfaquinoxaline, and sulfadimethoxine) in the sixth round of selection (as shown in Fig. 1). The ssDNA library was incubated with a mixture of the above analogs (same concentration as SMZ) at 25 °C for 1 h in 200 μL binding buffer.

Cloning, sequencing, and bioinformatics analysis

Since the recovery rate of ssDNA did not increase after the 7th round of selection, the ssDNA library recovered from the 7th round was amplified with unlabeled primers. The aptamers bound to SMZ were enriched, and the PCR products were cloned and sequenced by Sangon Biotech (Shanghai, China). The homology and secondary structure of candidate sequences were analyzed by DNAMAN and Mfold program (http://mfold.rna.albany.edu/?q=mfold/DNA-Folding-Form).

Determination of binding affinity, specificity, and limit of detection

To determine the binding affinities of the candidate aptamers, different concentrations of FAM-labeled aptamers (12.5, 25, 50, 75, 100, 150, and 200 nM) were incubated with 7 μL SMZ (4 μg/mL) in 200 μL 1× binding buffer at 25 °C for 1 h in the dark. The ssDNA/GO at different mass ratios (i.e. 1:24 and 1:700) was added to each analyte and incubated at room temperature for 20 min. The residual aptamers physically adsorbed to the GO was removed by centrifuging at 15,000 rpm for 10 min. The fluorescence intensity of the supernatant was measured by a Varioskan™ LUX Multimode Microplate Reader. A negative control without SMZ was also included to assess non-specific binding. The K_d value was estimated according to the $Y = B_{max} \times X/(K_d + X)$ equation from four experiments by nonlinear regression analysis using Origin 8.0 software (Northampton, MA), where X represents concentration of candidate aptamers, Y is the relative fluorescence intensity, and B_{max} is the maximum number of binding sites [37]. To evaluate the binding specificity of the aptamers to targets, 100 nM aptamers were incubated with SMZ or its different structural analogs (i.e., sulfameter sulfaquinoxaline, sulfadimethoxine, nitrofurantoin, ethanamizuril, and kanamycin) in 200 μL binding buffer. The target-bound aptamer was collected by centrifuging as described above, and the fluorescence

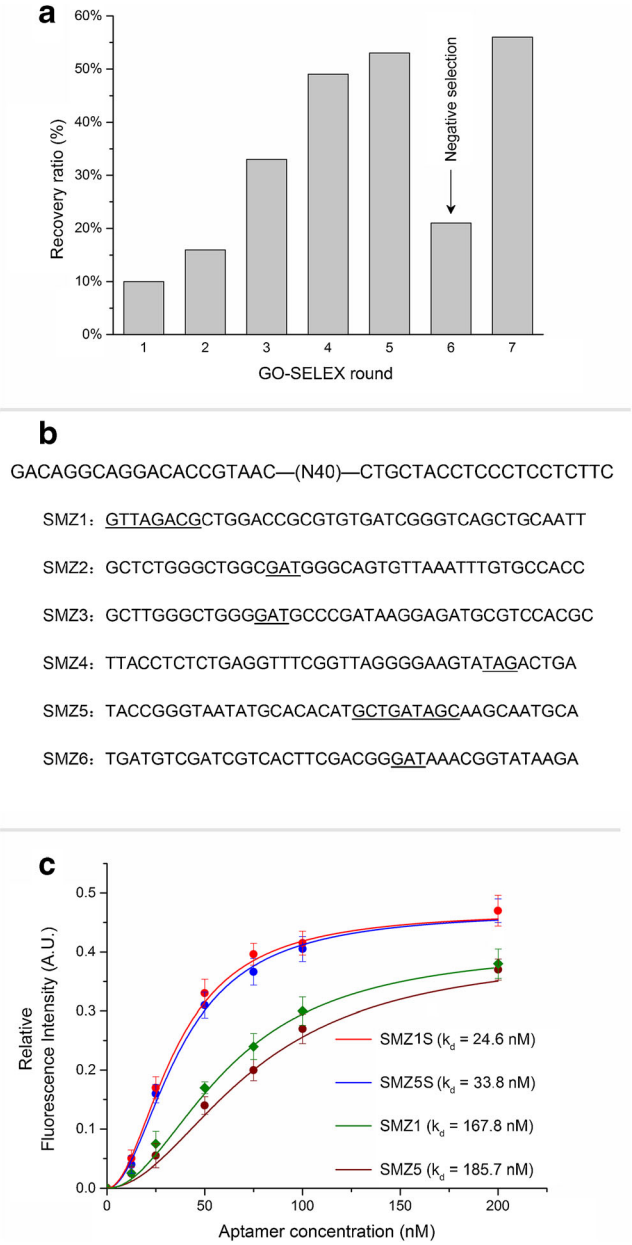

Fig. 2 (a) Recovery ratio of aptamers after each round of screening. (b) The sequences of the ssDNAs and SMZ1-SMZ6. The shared sequences are underlined in each ssDNA sequence. (c) The relative fluorescence intensity versus concentration of the FAM-labeled aptamers. The dissociation constants of aptamers were used to determine by nonlinear regression analysis. The relative fluorescence intensity is expressed as $(F - F_0)$, where F and F_0 are the fluorescence intensity obtained with the SMZ analyte and the blank sample, respectively

intensity was recorded. To determine the linear range and limit of detection (LOD), FAM-labeled aptamers (100 nM) were incubated with 0.4 to 500 ng/mL SMZ in 200 μL binding buffer in the dark at 25 °C for 1 h. The fluorescence intensity of the supernatant was measured at 520 nm ($\lambda_{exc} = 492$ nm) using Varioskan™ LUX. The LOD value was calculated as 3 ∗ SD/slope, where SD represents the standard deviation of the smallest point and the slope is determined from the fit graph [38].

Molecular docking of aptamer

To determine the possible binding mechanism between SMZ and SMZ1S aptamer (a truncated aptamer), the secondary and tertiary structures of the latter were first predicted using the Mfold program (http://www.bioinfo.rpi.edu/applications/mfold/; http://biophy.hust.edu.cn/3dRNA). The SMZ ligand was drawn using Chembio3D Ultra 14.0 software and its energy was minimized. Molecular docking was performed by AutoDockTools 1.5.6, and all hydrogen atoms and Gasteiger charges were added to SMZ1S in a rigid form. The entire aptamer molecule was covered by a tertiary structure grid box with grid points of 60 × 50 × 70 Å and a line spacing of 0.324 Å, and analyzed using PyMoL 2.3.2 software (http://www.pymol.org/).

Detection of SMZ in milk and egg samples

The GO-based aptasensor was used to test milk and egg samples spiked with SMZ. The milk and egg samples were previously confirmed using HPLC analysis [4], to be free of SMZ compounds. Specifically, to prepare the spiked milk samples, 10-mL milk samples were centrifuged at 14,000 rpm at 4 °C for 20 min, and the supernatants were diluted to 100 mL with binding buffer and filtered through a 0.22-μm porous film, and then spiked with different concentrations of SMZ. To prepare the spiked egg samples, eggs purchased from a local market were cracked and the contents were fully homogenized. Two grams of eggs was mixed with 4 mL ethyl acetate and shaken for 3 min. After centrifuging at 5000 rpm for 5 min at room temperature, the supernatant was dried at 80 °C in a water bath or under nitrogen. The residues were diluted to 500 μL with binding buffer, and spiked with different concentrations of SMZ. Then, the samples were tested by the aptasensor as described above.

Results and discussion

Optimization of the mass ratio of ssDNA library to GO

To determine the optimum GO mass ratio for aptamer selection by SELEX, we incubated ssDNA library and GO at various mass ratios, and analyzed the fluorescence intensity in the supernatant. As shown in Fig. S1 (see ESM), when the mass ratio between the ssDNA library and GO was 1:24, the fluorescence intensity in the supernatant approached to zero, indicating that the ssDNA library was almost completely adsorbed on GO. Therefore, ssDNA/GO ratio of 1:24 was used for subsequent experiments.

PCR optimization

Parameters used in PCR significantly affect the target affinity of the amplified sequence. Therefore, we tested six annealing temperatures (53 °C, 55 °C, 57 °C, 59 °C, 61 °C, and 63 °C) for 11, 13, 15, 17, 19, and 21 cycles in order to determine the optimum conditions for amplifying the candidate aptamers. As shown in Fig. S2a (see ESM), the highest fluorescence was obtained at the annealing temperature of 59 °C, and 15 amplification cycles resulted in the strongest band in gel electrophoresis (ESM Fig. S2b). In contrast, the electrophoretic bands were weaker for samples obtained under other conditions, indicating that the amount of enriched ssDNA obtained from 15 cycles was the largest. Therefore, the annealing temperature of 59 °C and 15 amplification cycles were selected as the optimal PCR conditions.

Selection of aptamers against SMZ

Efficient screening of high-affinity ssDNA from a random library depends on the removal of unbound or weakly bound ssDNA. Conventional aptamer screening methods involve

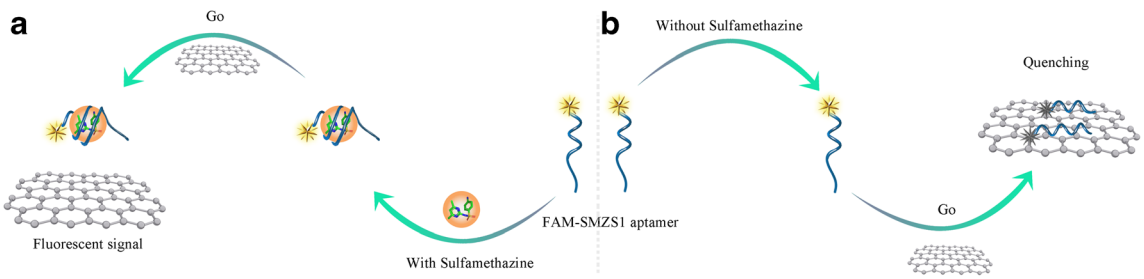

Fig. 3 Schematic illustration of the GO-based aptasensor assay for SMZ detection. Fluorescence signals (a) generation and (b) quenching

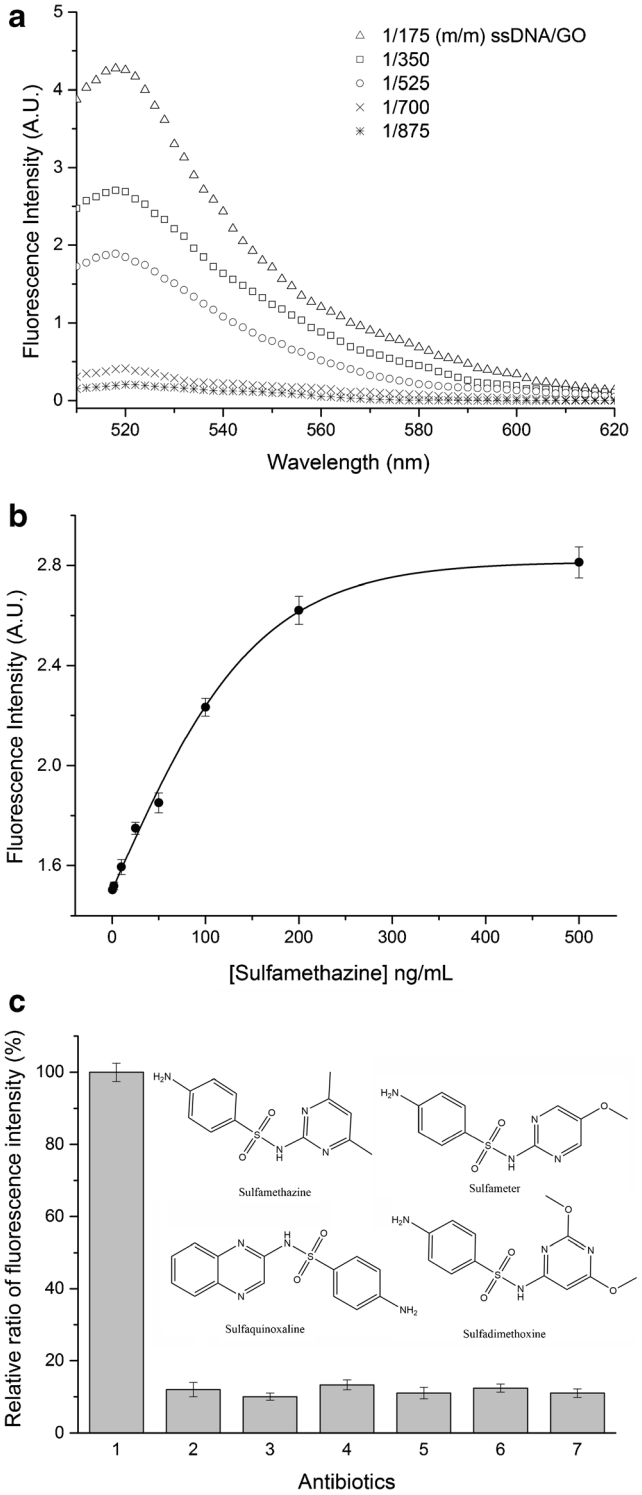

Fig. 4 (a) The fluorescence emission spectrum of SMZ1S aptamer as a function of ssDNA/GO mass ratio. (b) Fluorescence intensity (520 nm) of the aptasensor against 0.4 ng/mL to 500 ng/mL SMZ. (c) The fluorescence intensity of 1 μM different structural antibiotics is measured relative to sulfamethazine as the reference standard. (1) Sulfamethazine, (2) sulfameter, (3) sulfaquinoxaline, (4) sulfadimethoxine, (5) nitrofurantoin, (6) ethanamizuril, and (7) kanamycin

immobilization of the target or library on a solid substrate [20]. However, these methods require complex chemical reactions that would often alter the molecule structures of the targets, reducing the efficiency of aptamer screening as well as aptamer affinity [38]. In contrast, GO-SELEX is based on non-immobilized targets or libraries, and the target analytes are directly contacted with random ssDNA libraries, thereby improving aptamer screening. As shown in Fig. 1, the specific aptamers were identified by positive selection, magnetic separation, and counter selection. Positive selection was used in the early rounds to enrich for the high-affinity ssDNAs. Since the non-specific ssDNA strands were physically adsorbed on the GO surface by π-π stacking, the target-bound ssDNA in the supernatant could be easily amplified and digested for the next screening round. Magnetic separation was performed in the later rounds to remove complementary strands of DNA and non-specifically bound ssDNAs. The aptamers with high affinity and selectivity were enriched through multiple screening rounds. Subsequently, a counter screening was performed using three structural analogs of SMZ to further increase the specificity of the SMZ target aptamers.

The efficiency of each selection was determined by recovery rate of ssDNA. As shown in Fig. 2a, the recovery rate of target-bound ssDNA increased with each round, indicating an increase in the affinity between ssDNA and the target. However, in the sixth round, the recovery rate of the counter selection procedure decreased, likely due to binding of some ssDNA to the non-targe. After the sixth round of screening, the recovery rate plateaued at 55%, suggesting that binding of high-affinity and specific ssDNA to the target reached saturation. Therefore, ssDNAs recovered in the seventh round were identified, cloned, and sequenced. Finally, six SMZ binding aptamers—SMZ1, SMZ2, SMZ3, SMZ4, SMZ5, and SMZ6—with different random 40-mer sequences and two 20-mer conserved sequences were obtained and selected for subsequent experiments (Fig. 2b).

Optimization of aptamer structure

The secondary structures of the six candidate aptamers were predicted and analyzed by Mfold. As shown in Fig. S3 (see ESM), SMZ1, SMZ2, SMZ3, SMZ4, and SMZ5 shared conserved sequences in the loop sections such as "TAG," "GGTAG," "TAGGGGT," "ATAG," and "TAG," respectively, and base pairs in the stem portions such as "AT-CG-GC," "GC-CG," "GC-CG-CG," "AT-CG-TA," and "AT-GC-CG," respectively. Furthermore, all aptamers shared "TAG" sequence and "GC-CG" base pair, and SMZ1 and SMZ5 aptamers showed greater similarity between the random base sequences "CGTTAGACG" and "CGATAGTCG." Only SMZ1 and SMZ5 aptamers shared conserved "TAG" sequences in the loop sections, suggesting the notion that these regions may be involved in SMZ binding. Based on the

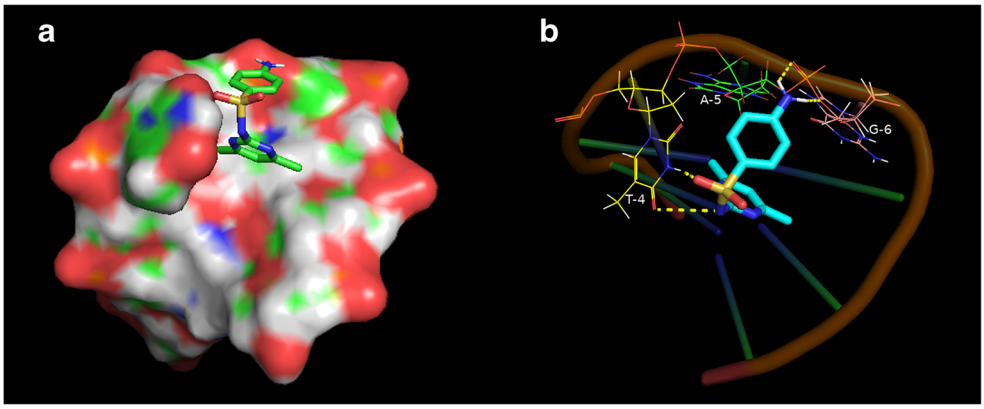

Fig. 5 Predicted binding between SMZ and SMZ1S as indicated by the (a) surface display diagram and (b) molecular docking

conservative structure and the putative model, both SMZ1 and SMZ5 were respectively truncated into the 9-mer sequences SMZ1S (base sequence CGTTAGACG) and SMZ5S (base sequence CGATAGTCG). It is noted that the binding affinity of SMZ1S (K_d = 24.6 nM) was the highest among all investigated (SMZ5S 33.8 nM, SMZ1 167.8 nM, and SMZ5 185.7 nM (Fig. 2c). Since SMZ1S had the highest affinity for SMZ, it was selected for subsequent experiments. The affinity of aptamer (SMZ1S, 9-mer sequences) in this work is 3 times compared with that reported aptamer (SA07, 96-mer sequences) previously [20].

Detection of SMZ by GO-based aptasensor

We next developed an aptasensor for quantifying SMZ by labeling the SMZ1S aptamer with fluorescent FAM. In the presence of SMZ, specific interactions between the fluorescently labeled aptamers and SMZ would release the fluorescently labeled aptamers from the GO, thus generating fluorescence signals proportional to the amount of SMZ present (Fig. 3a); in the absence of SMZ, physical adsorption of the fluorescently labeled aptamers to the GO through π-π stacking interactions would quench the fluorescent signal (Fig. 3b). We tested different ssDNA/GO mass ratios (1:175, 1:350, 1:525, 1:700, and 1:875) with 100 nM SMZ1S. As shown in Fig. 4a, the fluorescence intensity of the FAM label was almost zero and could not be restored by adding SMZ when ssDNA/GO mass ratio was 1:875. However, at the mass ratio of 1:700, the detection system not only showed high quenching efficiency in the absence of the target SMZ but also demonstrated changes in fluorescence intensity in responses to increasing SMZ concentrations. Therefore, a ssDNA/GO mass ratio at 1:700 was selected to construct the aptasensor. In addition, three different blocking agents were also tested, and the fluorescence intensity was the highest for buffer A as opposed to buffers B and C (ESM Fig. S4). Therefore, the result indicated that buffer A was the best reagent for the developed aptasensor.

The pharmacological parameters of the aptasensor were determined over 0.4–500 ng/mL SMZ in buffer A (Fig. 4b). The standard curve showed good linearity between 2 and 100 ng/mL SMZ, and the linear regression equation was $y = 0.0066x + 1.5259$. The limit of detection (LOD) for SMZ was 0.35 ng/mL. The specificity of the aptasensor for SMZ was assessed. As shown in Fig. 4c, none of the antibiotics interfered with SMZ detection, indicating high specificity of the SMZ1S aptasensor to SMZ.

Assessment of aptamer-SMZ interaction by molecular modeling

The aptamer binding sites were predicted based on the compatibility of the spatial structure, hydrogen bonding,

Table 1 Accuracy and precision of the developed fluorescence-based aptasensor for SMZ in egg and milk (n = 5)

Sample	Spiked (μg/kg)	Intra-assay		Inter-assay	
		Mean recovery (%) ± SD	CV	Mean recovery (%) ± SD	CV
Milk	10	100.6 ± 12.8	12.7	101.2 ± 8.9	8.8
	50	108.8 ± 6.8	6.3	106.7 ± 5.4	5.1
	100	94.4 ± 5.1	5.4	93.9 ± 6.8	7.2
Egg	10	103.1 ± 11.5	11.1	98.6 ± 7.9	8.0
	50	107.9 ± 9.8	9.1	104.9 ± 6.7	6.4
	100	96.5 ± 5.3	5.5	93.9 ± 4.9	5.2

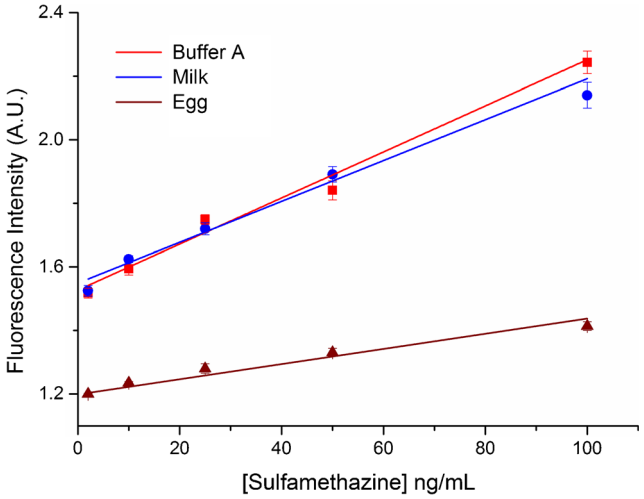

Fig. 6 The linear fit of the fluorescence intensity of the aptasensor at 520 nm in buffer A, and SMZ-spiked milk and egg samples

electrostatic attraction, or a combination of these parameters, and simulated by molecular docking. As shown in Fig. 5a, SMZ was located inside the loop of aptamer SMZ1S, suggesting that the aptamer-ligand binding was closely related to the three-dimensional surface structure of the former. Furthermore, the hydrogen atoms of the amino groups of SMZ formed hydrogen bonding with the phosphodiester "O" of nucleotide G-6, and the bond lengths were 2.1 Å and 2.2 Å (Fig. 5b). In addition, hydrogen bonding also formed between the oxygen atom of the SMZ sulfonamide and the hydrogen atom of the nucleotide thymine-4 (1.9 Å), and between the hydrogen atom of SMZ sulfonamide and the oxygen atom of nucleotide T-4 (3.1 Å). The binding free energy of SMZ1S and SMZ was calculated to be − 3.86 kcal/mol. Taken together, the molecular recognition of targets by the aptamer is the result of multiple binding interactions.

Detection of SMZ in egg and milk samples

The efficacy of the aptasensor was tested on milk and egg samples spiked with different concentrations of SMZ in order to demonstrate its efficacy in complex biological samples. As shown in Fig. 6, the fluorescence intensity of the test samples gradually increased with increasing SMZ concentrations. The respective LODs for buffer A, milk, and egg samples were 0.35, 0.62, and 1.18 ng/mL, respectively. Furthermore, the average fluorescent recovery rates ranged from 93.9 to 108.8% with the coefficients of variation (intra-assay and inter-assay CV) less than 12.7% (Table 1), which confirmed the accuracy and precision of the aptasensor within an acceptable range.

Conclusions

We successfully screened, identified, and optimized high-affinity SMZ-specific aptamers through GO-SELEX. The truncated aptamer SMZ1S (sequence 5′-CGTTAGACG-3′) showed the lowest K_d of 24.6 nM and higher specificity to SMZ. The molecular docking provided important information and reasonable explanations for the interactions of SMZ molecules and aptamers. Under optimized conditions, the FAM-labeled fluorescent SMZ1S aptasensor detected SMZ with high accuracy (93.9–108.8%), reproducibility (CV 5.1–12.7%), and sensitivity (LOD 0.35–1.18 ng/mL), and is therefore a promising tool for the sensitive and accurate detection of SMZ residues in animal-derived foods.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animals This article does not contain any studies with human subjects. All animal experiments described in the present study were performed in adherence to Chongqing Normal University animal experiment center guidelines and approved by Animal Ethics Committee of the Chongqing Normal University.

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