Published in final edited form as:
Mikrochim Acta. 2014 April ; 181(5-6): 479–491. doi:10.1007/s00604-013-1156-7.

Recent trends in SELEX technique and its application to food safety monitoring

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Abstract

The method referred to as "systemic evolution of ligands by exponential enrichment" (SELEX) was introduced in 1990 and ever since has become an important tool for the identification and screening of aptamers. Such nucleic acids can recognize and bind to their corresponding targets (analytes) with high selectivity and affinity, and aptamers therefore have become attractive alternatives to traditional antibodies not the least because they are much more stable. Meanwhile, they have found numerous applications in different fields including food quality and safety monitoring. This review first gives an introduction into the selection process and to the evolution of SELEX, then covers applications of aptamers in the surveillance of food safety (with subsections on absorptiometric, electrochemical, fluorescent and other methods), and then gives conclusions and perspectives. The SELEX method excels by its features of in vitro, high throughput and ease of operation. This review contains 86 references.

Keywords

SELEX; Aptamer; Food safety; Rapid detection; Biosensor

Introduction

Systemic evolution of ligands by exponential enrichment (SELEX) is firstly described in 1990, which is used as an in vitro selection tool to discover a new kind of nucleic acid ligands, named aptamers. Ellington and Szostak [1] firstly reported the selection of RNA aptamer which can specifically combine with small organic dyes while Tuerk and Gold [2] also described the selection of aptamer against T_4DNA polymerase. The name "aptamer" is originally derived from the Latin expression "aptus" (to fit) and the Greek word "meros" (part). Intrinsically, aptamers are short oligodeoxynucleotides which can adopt specific three dimensional conformations to combine with target analytes. Till now, according to most of the reported achievements, the targets of aptamers rang from small ions (e.g. Zn^{2+} [3]), small organic compounds (e.g. organic dyes [4], neutral disaccharides [5], and antibiotics [6]) to large molecules such as glycoproteins (such as CD4 [7]) or even complex targets (e.g. the whole living cells [8, 9]).

Currently, the aptamers have been proposed as the potential substitution to conventional antibodies due to their similar recognition properties and other superior properties. In details, aptamers can even offer several advantages over traditional antibodies that make them very promising for practical analytical applications. Firstly, aptamers can be selected through vitro selection that are independent of immune animals, while antibodies are obtained by making use of animals or cell lines, which is a complicated and time-consuming process; Secondly, aptamers are more stable than antibodies, since they can recover their native active property and conformation after denaturation. On the contrary, antibodies would undergo irreversible denaturation under the condition of high temperature or some

other extreme or harsh conditions; finally, aptamers could be more easily chemically prepared and modified with other active groups in scale with low cost. Since aptamers show so many advantages over traditional antibodies, they are used as attractive substitutions to antibodies and widely applied in the field of food safety monitoring.

In recent years, aptamer-based bioassay has been extensively adopted in clinical, medical, environmental monitoring, and food analysis. Several classic aptamers, including Pb^{2+} [10], Hg^{2+} [11], ochratoxin A [12], and tetracycline [13] have been successfully selected with SELEX and applied in food safety monitoring [14]. With respect to these aptamer-based applications, they could be divided into affinity chromatography [15], capillary electrophoresis [16], mass spectrometry [17] and biosensors [18] based methods et al.

In this review, we aim at illustrating the recent development of SELEX in bioassays and its specific applications in food safety monitoring. We especially summarized the aptamer-based biosensors in the field of food safety focused on the aptamer-based colorimetric methods, electrochemical methods and fluorescence methods. Finally, we also put forward some perspectives of aptamer-based methods for rapid and ultrasensitive food safety monitoring.

Selection process and evolution of SELEX

Aptamer selection process, also called SELEX (systematic evolution of ligands by exponential enrichment), is first discovered almost simultaneously by two independent research groups of Ellington and Tuerk in 1990, respectively. It is a technique for screening target oligonucleotide probes from large libraries of oligonucleotides (RNA or single-stranded DNA) by an iterative in vitro selection process and amplification by polymerase chain reaction (PCR) of selected sequences. SELEX has been adopted as an important research tool for recognition probe screening and extensive efforts has been further devoted for improvement of screen efficiency. The detailed basic steps of SELEX process are presented in Fig. 1 [19]. Specifically, SELEX process includes (1) the incubation of target molecules with the random sequence pools, (2) and the subsequent separation of unbound oligonucleotides and the elution of bound oligonucleotides, (3) then PCR amplification of bound aptamers. The step-by-step protocols of SELEX are as follows: target molecules are incubated with the random library for a period of time. Unbounded oligonucleotides are removed by several binding buffer washing steps. After the unbounded oligonucleotides are separated, the target-bound oligonucleotides are eluted by heating or several elution buffer washings. The eluted sequences are amplified by PCR to form the starting pool for the next round of selection. The entire process cycles are then repeated for several rounds, in general, as the numbers of selection rounds are increased, a corresponding steady increase in binding affinity of the aptamer candidates is observed, and about 8–15 rounds of selection and amplification are needed [20].

Besides, several improvements are introduced into SELEX in order to reduce the whole selection time (automated-SELEX [21]), or improve the aptamer selectivity (blended-SELEX [22], counter-SELEX [23], and negative-SELEX [24]). It usually takes from weeks to months to obtain the ideal aptamers with high affinity against the targets by the

conventional in vitro selection procedures due to the repetitive, and poorly adapt to high-throughput applications. Following, the automated SELEX process has been recently explored by Cox and Ellington in 2001 [21]. The automated workstations can achieve eight selections in parallel, and would complete approximately 12 rounds of selection in just 2 days, which improve the screening efficiency dramatically and further widen the application field of SELEX. Besides, this platform could give an additional 12-fold increase in throughput, which further greatly improves the selection efficiency [21]. In 2003, a selection strategy named Cell-SELEX is designed to the whole cells [25] and a glioblastoma-derived cell line-U251is adopted as the target. Cell-SELEX allows the oligonucleotide pools to recognize target cells, especially cancer cells and pathogenic bacteria. In 2004, Mendonsa and Bowser adopted capillary electrophoresis-SELEX (CE-SELEX), which used capillary electrophoresis as an alternative separation method [26]. Sequences bound to the targets migrate through the capillary at a certain voltage different from those not bound to the targets, allowing binding sequences to be isolated from nonbinding sequences. Advantages of CE-SELEX include increased separation power, reduced nonspecific binding, and ability to perform the selection in free solution system. Benefit from these advantages, high affinity aptamers can be obtained in only 2–4 rounds of selection instead of the 8–12 rounds needed by conventional selections [26]. In addition, SELEX process integrated with micro-magnetic separation has also been reported recently [27]. For example, single stranded DNA aptamers that bind to oxytetracycline (OTC) have been successfully selected with the help of OTC-coated magnetic beads as immobilization matrix [28]. John G. Bruno et al. have adopted a route using magnetic beads in aptamer selection and related detection of cholera bio-toxin [29]. Use of magnetic beads for affinity separation of aptamers can realize several advantages. Firstly, the sample volumes are small (50–100 μL). Secondly, with the use of magnetic beads, bound oligonucleotides could be directly separated by magnetic separation rather than column separation with a high efficiency in partitioning step. Finally, PCR amplification can be directly carried out on the surface of the magnetic beads, which is one important research field in nanobiotechnology.

Aptamers usually vary in length from 25 to 90 bases, and their typical structural motifs can be classified into stems [30], internal loops, purine-rich bulges, hairpin structures, hairpins, pseudoknots [31], kissing complexes [32], or G-quadruplex structures [33] et al. Most of the aptamers can bind to a wide variety of targets through their adaptive conformational changes and three-dimensional folding. There have been many reports of screening of aptamer against the targets in the field of food safety monitoring. The typical targets include the bio-toxins of the mycotoxins ochratoxin A [36], botulinum neurotoxin [37], and fumonisin B [38], bacterial pathogens of salmonella typhimurium [42], listeria monocytogenes [43] and staphylococcus aureus [44], as well as antibiotics of tetracycline [49], kanamycin B [47]. For extensive comparisons and studies, detailed aptamers that are involved in food safety are summarized in Table 1.

Application of aptamers in food safety monitoring

Detection of hazards in food samples, such as heavy metals ions, bio-toxins, illegal additives, pathogenic microorganisms and antibiotics, is of great importance and continuous to be a significant challenge. Traditional analytical techniques including surface plasmon

resonance (SPR) [55], high performance liquid chromatography (HPLC) [56], capillary electrophoresis (CE) [57], antibody-based enzyme linked immunosorbent assay (ELISA) [58], are laborious, time-consuming and expensive. To overcome these disadvantages, aptamer-based assays have been paid great attention and developed for on-site and rapid detections due to their great advantages of high sensitivity, selectivity and stability. Some schematic diagrams of aptamer-based assays widely used in food safety monitoring are presented in Fig. 2. And the summary of hazards in food safety monitoring is showed in detail in Table 2. Furthermore, some important and intensive studied aptamer based methods for on-site, rapid and ultrasensitive detections in food safety are summarized as follows:

Absorptiometric methods

Absorptiometric methods (also called colorimetric methods) based on colloidal gold nanoparticles (DNA/AuNPs) have attracted much attention in recent years due to the convenience of visual observation, easy operation and on-site detection properties. DNA oligonucleotides are used as recognition units, while gold nanoparticles usually serve as labeling tags for their unique optical properties. Colorimetric methods have been widely used in detection of heavy metals ions, small molecules chemical residues, antibiotics and bio-toxins.

Monitoring of toxic metals ions is an important issue since these contaminants have server adverse effects on human health. Although the conventional detection techniques, such as inductively coupled plasma mass spectrometry (ICP-MS), are adopted in most current protocols for detection of heavy metal ions. However, these instrument-based methods are expensive, complicated, professional personnel needed and not suitable for on-site detection. Aptamer based colorimetric methods have great advantages over the traditional methods. Su et al. [59] and coworkers have reported colorimetric detection of lead ions (Pb^{2+}) using glutathione functionalized gold nanoparticles. As shown in Fig. 3, aggregation of glutathione modified gold nanoparticles (GSH-AuNPs) in the presence of Pb^{2+} is induced by the chelating effects, yielding both a substantial shift in the plasmon band energy to longer wavelength and a red-to-blue color change [59]. This sensor can be used for detecting Pb^{2+} with a minimum detectable concentration of 100 nM. Yang [60] and co-workers have presented the colorimetric detection of mercury ions (Hg^{2+}) based on DNA oligonucleotides and unmodified gold nanoparticles. DNA oligonucleotides with T-T mismatches retain random coil in the absence of Hg^{2+} ions. These ssDNA units could effectively protect AuNPs from salt-induced aggregation. While in the presence of Hg^{2+} ions, DNA oligonucleotides could form duplex structures by the specific T-Hg^{2+}-T coordination chemistry, inducing the release of ssDNA from the surface of AuNPs. Without the protection of the ssDNA, the AuNPs aggregated under the high-salt condition and achieved a color change from red-wine to dark blue, which could be adopted as the quantitative indicator for Hg^{2+} sensing. A sensitive linear range from 0 to 5 μM and a detection limit of 0.5 μM for Hg^{2+} ions are easily obtained. Xie [61] and coworkers have designed a triple-channel optical signal probe for Hg^{2+} detection. Without the Hg^{2+}, the T-rich ssDNA probe is attached on the surfaces of gold nanoparticles with the protection effect, maintaining the red color of the solution. In the presence of target Hg^{2+}, the ssDNA forms the specific T-Hg^{2+}-T configuration, which induces a color change from original red to dark blue. Under

the optimum conditions, the developed Hg^{2+} sensing system exhibits a dynamic response range from 50 nM to 5 mM with a detection limit of 30 nM. In addition, a DNAzyme probe has also been used for detection of Hg^{2+} [62]. The exhibited peroxidase-like activity of T-rich G-quadruplex DNA could be inhibited in the presence of Hg^{2+} ions through Hg^{2+}-mediated T-T base pairs. In the presence of a coordination cation K^+, T-rich DNA folds into a G-quadruplex structure and bind with hemin to form the hemin-G-quadruplex DNAzyme. After addition of Hg^{2+}, the folded G-quadruplex of T-rich DNA is subjected to unfolding due to the Hg^{2+}-mediated formation of T-Hg^{2+}-T mismatched pairs. This gives rise to a decrease in the DNAzyme activity accompanied by a color change in the TMB-H_2O_2 reaction system, enabling the detection of aqueous Hg^{2+} ions with visual observation with LOD of 100 nM. In short, it could be come to the conclusion that these aptamer based methods could meet the requirement of rapid, easy-operation and on-site detections. All these aptamer based rapid screening methods for heavy metal ions have definitely supplied us with valuable experience and potential directions for future research of heavy metal detection.

DNA/AuNPs based colorimetric methods can also be employed for detection of small organic molecules. Ochratoxin A (OTA), a type of mycotoxin with small molecular weight, is mainly produced by fungal species in aspergillus and penicillium genera. According to the previous studies, OTA is hepatotoxic, nephrotoxic, neurotoxic, teratogenic and mutagenic to most of the mammalian species. Routine and rapid screen of OTA in food samples are of great importance for human health and public safety. Yang [63] and coworkers have developed an aptasensor for biosensing of ochratoxin A (OTA) using aptamer-DNAzyme hairpin as bio-recognition element as illustrated in Fig. 4. The structure of probe is divided into three parts, one is the horseradish peroxidase (HRP)-mimicking DNAzyme, one is the OTA specific aptamer sequences and the left one is a blocking tail captures in the stem region of the hairpin. In the presence of OTA, the hairpin is broken due to the formation of the aptamer-OTA complex activating the HRP-mimicking DNAzyme. The activity of this DNAzyme is linearly correlated with OTA concentration up to 10 nM and a limit of detection of 2.5 nM. They also carried out the research about OTA detection using an aptamer-based colorimetric biosensor with unmodified gold nanoparticles as the indicator [64]. In the absence of OTA, aptamer to OTA could be easily adsorbed onto the surface of AuNPs, enhancing the stability of AuNPs against salt-induced aggregation. In the presence of OTA, the conformation of aptamer changes from random coil to G-quadruplex structure losing the ability to protect AuNPs. Upon the addition of salt solution, the reaction solution achieved a color change from red to blue in the range from 20 to 625 nM and 20 nM was obtained as limit of detection. Wang [65] and co-workers have constructed an aptamer-based chromatographic strip assay for sensitive toxin detection. As shown in Fig. 5, the principle of the designed aptamer-based strip is based on the competitive reaction between the DNA probe 1 (test line) and target OTA to combine with aptamers. In the presence of OTA, it would combine with aptamer-AuNPs probe, decreasing the number of aptamer-AuNPs that could hybridize with the DNA probe 1 on the test line. LOD of the aptamer-based strip for OTA is as low as 1 ng mL^{-1} by visual inspection. While the LOD of semi-quantitative detection could be as low as 0.18 ng mL^{-1} by using scanning reader, which is quite better than that of the antibody-based strip and is even comparable to ELISA.

Antibiotics are widely adopted to kill or inhibit microorganisms in food products to maintain the quality or elongate the shelf-life, while the abuse of them would result in various side effects in human and even the appearance of super bacteria with tolerance to related antibiotics [66, 67]. Song et al. [68] have carried out a gold nanoparticle-based colorimetric method for kanamycin using aptamer probe. Similarly, aptamers are assembled on the surface of AuNPs and prevent the aggregation upon salt introduction. In the presence of kanamycin, the aptamers are departed from the AuNPs, which induces the aggregation of AuNPs and exhibits a color change from red to purple or blue. Using this aptamer, kanamycin is detected down to 25 nM by the colorimetric method. Melamine is reported to be illegally added into milk products for the aim of improving the content of protein. Although the low toxicity of melamine itself, it could induce the formation of insoluble crystals in combination with cyanuric acid in digestion process, which further lead to the kidney stones, the kidney failure of infants and children, and even to death [69]. Liang et al. [69] has developed a highly sensitive aptamers-AuNPs catalytic resonance scattering spectral assay for melamine. The aptamers (ssDNA) are used to label AuNPs as the recognition probe for melamine. Upon addition of melamine, it interacted with the aptamer to form bigger aptamers-AuNPs-melamine aggregations, increasing the resonance rayleigh scattering (RRS) intensity at 566 nm greatly. The increased RRS intensity is linear to melamine concentration in the range of 1.89–81.98 μg L^{-1} with a detection limit of 0.98 μg L^{-1} [69]. In all, the absorptiometric methods for food safety monitoring are all with the priorities of rapid, easy-operation and without the expensive instruments. These advantages make them more competitive under the condition of mass detection requirements compared with the traditional protocols. However, the stability and the repeatability of the absorptiometric methods should also be paid great attention to make them more practically applicable.

Electrochemical based methods

Nowadays, aptamer-based electrochemical sensors appear to be a promising alternative for detection of hazards in various food products including heavy metals ions [70, 71], tetracycline antibiotics [72] and so on. Aptamer-based electrochemical biosensors have attracted considerable attention because they could provide some extraordinary properties including simple, fast, and ultrasensitive determinations with low cost compared with other analytical strategies.

In 2006, the Plaxco team [70] reported the electrochemical methods for detection of Pb^{2+} via assembled DNAzyme on electrode as showed in Fig. 6. The electrochemical sensor consists of a methylene blue (MB) modified DNA strand which is chemically absorbed to a gold electrode via a 5′ terminal thiol. In the presence of Pb^{2+}, MB transfers electrons to the electrode, and then produces the electrical signal. The detection limit of the electrochemical sensor toward Pb^{2+} is as low as 62 ppb (0.3 μM). Liu [73] and coworkers utilized an impedimetric DNA sensor for detection of Hg^{2+} and Pb^{2+} at the same time. This electrochemical sensor includes a pair of DNA probes, a Pb^{2+}-specific DNAzyme and a substrate strand containing Hg^{2+}-specific oligonucleotide. Both conformational changes including Hg^{2+} induced duplex-like structure and Pb^{2+} induced cleaving of substrate at the RNA site are investigated by electrochemical impedance spectroscopy (EIS). As a result,

these mentioned aptamer conformational changes lead to the decreased EIS results, which allows selective detection of Hg^{2+} and Pb^{2+} with the detection limit of 1 pM and 0.1 pM, respectively. Furthermore, this developed method was applied to detect Hg^{2+} and Pb^{2+} simultaneously in human serum and river water samples by using nontoxic masking agents. Our group [74] has also fabricated an aptamer-based electrochemical “signal-off” sensor of OTA. The aptamer of OTA is immobilized on the surface of the electrode through hybridization with a linker DNA. A gold nanoparticle (AuNP)-functionalized DNA probe, complementary to the aptamer, is used to amplify the final sensing signal. In the sensing system, the target OTA competes with both the linker DNA and AuNP-functionalized DNA to combine with the aptamer, influencing the amount of dsDNA on the surface of the electrode. Methylene blue (MB), used as the electrochemical redox tags, is proportional to the amount of the dsDNA. Therefore, the redox currents of the electrochemical probe MB are proportional to the amount of OTA in the solution. The developed electrochemical sensor is also used to detect OTA in real wine samples. Recently, our group [75] has further fabricated a novel electrochemical aptasensor for detection of OTA using rolling circle amplification (RCA) as signal enhancement strategy. The primer for RCA is designed to compose of a two-part sequence, one part of the aptamer sequence directs against OTA while the other part is complementary to the capture probe on the electrode surface. In the presence of target OTA, the primer, originally hybridizes with the RCA padlock, is replaced to combine with OTA. This induces the inhibition of RCA and decreases the OTA sensing signal obtained with the electrochemical aptasensor. Under the optimized conditions, ultrasensitive detection of OTA is successfully achieved with a LOD of 0.065 ppt (pg/mL), which is much lower than previously reported.

Tetracyclines, the residues of which are widely found in many foods including meat, milk, honey, and chicken’s egg, can reduce the affinity of prokaryotic tRNA by strong binding on the 30S ribosomal subunit. Traditional detection methods for tetracycline are often time-consuming and expensive, which are not suitable for rapid and high throughput screening. Kim [72] and coworkers developed an aptamer-based electrochemical sensor for tetracycline detection. The biotinylated ssDNA aptamer of tetracycline is immobilized on a streptavidin-modified gold electrode. The recognition and binding process of tetracycline to aptamer is monitored and analyzed by cyclic voltammetry and square wave voltammetry, respectively. The developed sensor in this study is unique due to the fact that it can specifically distinguish tetracycline with a very broad detection limit (10 nM–10 μM), which could be chosen as the reference for sensor development in the future research. Kim et al. [35] has studied an electrochemical method for detection of 17β-estradiol. The selected ssDNA aptamer is immobilized on a gold electrode through the specific biotin-streptavidin interaction. When 17β-estradiol interacts with the DNA aptamer, the redox current is decreased due to the interference of bound 17β-estradiol with the electron flow produced by a redox reaction between ferrocyanide and ferricyanide. Using this method, 17β-estradiol could be well determined even in complicated agricultural products.

Fluorescent methods

Fluorophores have been widely used as signal generators in rapid and ultrasensitive detections. The fluorescence emission of the dyes can be adjusted by the changing the

distance with its quenchers or donors through conformational changes of aptamers linked with them. The aptamer-based fluorescent biosensors are promising assays for biological samples, suggesting their potential applications in biomedical researches. Herein we will focus on the developed aptamer-based fluorescent biosensors for food safety monitoring.

Nie [76] and coworkers have adopted a DNAzyme-based flow cytometric method for detecting Pb^{2+} ions as illustrated in Fig. 7. The Pb^{2+}-dependent GR-5 DNAzyme is labeled with a carboxyfluorescein (FAM) as the fluorescence reporter and a biotin for the conjugation with the streptavidin-functional magnetic beads (MBs) at the 5′ and the 3′ termini, respectively. The substrate strand is labeled with quencher Dabcyl at both ends. The DNAzyme combines with MBs and then hybridizes with substrate strand. In the presence of Pb^{2+}, the double quencher-labeled substrate strands are cleaved and disassociated from the DNAzyme. As a result, the FAM is no longer quenched by Dabcyl, resulting in the fluorescence recovery. The increased fluorescence intensity is linear to Pb^{2+} in the range of 1.0 nM to 100.0 nM with a LOD of 0.98 $\mu g \cdot L^{-1}$. Li [77] and coworkers have developed a fluorescent detection method for Pb^{2+} ions through the catalytic property of induced DNAzymes structure. The DNAzyme could act as a cofactor to catalyze H_2O_2-mediated oxidation of Amplex Ultra Red (AUR), resulting in increased fluorescent intensity. Under the optimal conditions, the DNAzyme-AUR probe is highly sensitive (LOD 0.4 nM) and selective (by at least 100-fold over other metal ions) toward Pb^{2+} ions with a linear detection range from 0 to 1000 nM. This is an extraordinary results compared with other traditional immunoassay methods without tedious sample treatments and long detection period.

An aptamer-based assay is also used for detection of aflatoxin B1 (AFB1). AFB1 is the predominant and most toxic hazards in cereal foods. Shim [78] and coworkers have reported a chemiluminescence competitive aptamer assay for detection of AFB1 in corn samples. The assay is conducted on traditional white 96-microtitre well plate. The AFB1 aptamer is linked with HRP-DNAzymes that produces sufficient chemiluminescence (CL) values when binding to AFB1-ovalbumin (OVA). In the absence of AFB1, the AFB1 aptamer combines with AFB1-OVA and produces chemiluminescence with the adding of luminol. However, in the presence of AFB1, the AFB1 aptamer combines with AFB1 and releases from the plate, decreasing the intensity of chemiluminescence. This aptamer assay has exhibited a wide dynamic range from 0.1 to 10 $ng \cdot mL^{-1}$and showed a LOD of 0.11 $ng \cdot mL^{-1}$. This sensitivity of this aptamer based methods could meet the requirement of AFB1 detection as the ELISA methods. However, the multi-step operations are still needed, which inhibit the application in the field of on-site detection. Future research should be focused on the simple and rapid detection with the excellent sensitivity for practical on-site detection of AFB1.

Other functional nanomaterials based fluorescent methods were also extensively studied. For example, PVP-coated graphene oxide for selective determination of ochratoxin A via fluorescence quenching was reported in 2011 by Wang and co-workers [79]. In the presence of target OTA, it induces the conformational change of the aptamer, leading to the formation of anti-parallel G-quadruplex, which is resistant to adsorption onto the larger planar surface of graphene oxide. Therefore, the fluorescent intensity is measured as a function of OTA concentration correspondingly. As low as 18.7 nM ochratoxin A can be detected through this protocol. It is also suitable for measuring OTA in real food samples such as red wines.

Quantum dot (QD)-labeled aptamer has been adopted as the novel fluorescent tags for detection of OTA. Previously, our group has reported a simple fluorescent strip biosensor based on QDs labeling technology which could meet the requirement of rapid toxin screening and monitoring. In detail, one of the designed DNA probe in our strategy, containing the complete complementary sequence to the aptamer was adopted as the probes on the test line of strip. In the presence of target OTA, the AuNPs labeled aptamer would preferably to bind with OTA instead of the complementary probes on the test line, which induced the fluorescent intensity decrease of test line. Based on the fluorescent intensity of the immobilized QDs in the test and control line, qualitative and quantitative detection of target OTA was successfully achieved [80]. Sara et al. [51] has reported an RNA-aptamer-based assay for the analysis of malachite green residues in fish tissue. It is the first report using an RNA aptamer for the development of a diagnostic method of chemical residues in food samples. The selected RNA aptamer can be used as an alternative recognition probe. The fluorescence of malachite green could be increased upon binding of the aptamer with the cognate ligand. Then the concentration of malachite green could be quantitatively determined according to the variations of the fluorescent intensity. Lu et al. [81] has reported a label-free fluorescent aptamer sensor based on regulation of malachite green (MG) fluorescence. The sensor contains free MG, adenosine (ATP) aptamer next to a MG aptamer, and a bridging strand that partially hybridizes to both adenosine aptamer and MG aptamer. MG is prevented to bind with MG aptamer by hybridization. In the presence of ATP, ATP aptamer binds with ATP, hybridization structures are opened. Then MG aptamer binds with MG and exhibits high fluorescence intensity. Under this protocol, the quantity of MG could be measured based on the intensity of fluorescent. Anyway, the fluorescent methods are with the priorities of rapid kinetics, easy measured pattern and satisfied sensitivity. Combined with the new recognition probe, aptamer, the fluorescent should be paid much more attention in the development of novel methods for on-site, rapid and ultrasensitive monitoring of food safety.

Other related methods

Surface plasmon resonance spectroscopy (SPR) is a surface-sensitive label-free technique that provides detailed information about the affinity and the kinetics of biomolecular interactions with high sensitivity. As a very easy operated protocol, SPR-based methods have been widely adopted for food safety monitoring. Gilad Pelossof et al. [82] has reported a surface plasmon resonance (SPR)-based sensor for detection of Pb^{2+} ions by using Pb^{2+}-dependent DNAzyme and hemin/G-quadruplex as the label. The ssDNA 2 in the reference contains two parts: one is G-quadruplex sequence and the other is a ribonuclease-containing substrate of the DNAzyme. The ssDNA 1 is Pb^{2+}-dependent DNAzyme sequence that forms duplex structures with the two parts of 2 and generates the sequence-specific loop for binding Pb^{2+} ions. The duplex structures are assembled on Au electrodes. In the presence of Pb^{2+} ions, the Pb^{2+}-dependent DNAzyme cleaves the substrate, leading to the separation of the complex and the self-assembly of the hemin/G-quadruplex on the Au substrate. In the sensing platform, the Pb^{2+} ions are analyzed by following the dielectric changes at the surface as a result of the formation of the hemin/G-quadruplex label using SPR. A detection limit of 1 pM could be achieved easily. Noemí de-los-Santos-Álvarez [83] has utilized a SPR sensor for detection of neomycin B with modified RNA aptamer. In this research, the

competitive recognition model was used for detection of neomycin B. The interaction between 2′ O-methylated RNA aptamer and neomycin B has been characterized by using SPR and electric impedance measurements. A range from 10 nM to 100 μM is obtained for the quantitative analysis of neomycin B. To the best of our knowledge, that is the first report of SPR-based aptasensor for small molecule detections. Besides, various novel aptamer-based approaches have been raised for rapid detection of different pathogens [84]. Ohk et al. [43] has reported an antibody-aptamer dual functionalized fiber-optic biosensor for specific detection of listeria monocytogenes (L. monocytogenes) in food samples. Aptamer-A8, the selected aptamer specific for internalin A, an invasin protein of L. monocytogenes, is applied in the development of fiber-optic sensor. Meanwhile the antibody against Listeria, P66 was also adopted in the sandwich format based protocol for L. monocytogenes. The biotinylated antibody was immobilized on optical fiber for capture of the target while the fluorescent tag labeled aptamer for signal reporting. This aptamer and antibody based biosensor could achieve detection of pathogenic Listeria in pure culture medium or in the mixture with other bacteria at a concentration of approximately 10^3 CFU mL^{-1}. Furthermore, this aptamer-based biosensor was also successfully applied in detection of L. monocytogenes from artificially contaminated (initial inoculation of 10^2 CFU 25 g^{-1}) ready-to-eat meat products including sliced beef, chicken and turkey with 18 h of enrichment [43]. Of note, the enrichment step is still needed for target bacterium detection in real food samples by the reported aptamer-based methods, which is the main obstacle for rapid and on-site screening. Instead, some other functional materials including sample pre-treating materials and magnetic nanoparticles could be the important supplemental techniques for improving the detection efficiency. And the research in this direction should be paid great attention.

Lee et al. [85] have developed a sensitive aptamer based method to detect Escherichia coli (E. coli) with immunomagnetic separation and real-time PCR amplification of aptamer. The scheme of the sensing principle is illustrated in Fig. 8. The three main steps are included in the detection process. Firstly, the target E. coli were captured by antibody-conjugated magnetic beads; secondly, the RNA aptamers are bound onto the surface of captured E. coli in a sandwich model; finally, the heat-released aptamers are amplified by using real-time reverse-transcriptase-PCR (RT-PCR). And a wide dynamic range from 10^1 to 10^7 CFU mL^{-1} of E. coli with the satisfied achievement was shown in this research. Our research team also developed a magnetic beads based screen method for Salmonella O8 and application of the selected aptamer in rapid fluorescent determination of Salmonella O8. The results indicated that the selected aptamer could be used to develop other rapid detection methods for Salmonella with the high specificity [86].

Conclusions and prospective

The SELEX technology is a promising tool for screening of aptamer against various target analytes and is essential for the development of new aptamer-based analytical methods. More importantly, the SELEX science, in both the breadth and width, is still under continuous evolution over the last two decades. Aptamer-based biosensors have been extensively applied in many fields including food safety monitoring for the significant better performance compared with the antibodies-based methods. Although, in theory, any targets

of interest could be selected with the specific aptamer, the rapid detection and diagnostic market is still dominated by the antibody-based methods. The possible reason for this phenomenon can be attributed to two aspects: 1) the categories of the target analytes with the specific aptamer are still limited, which is the main factor inhibit the wide application of aptamer based methods; 2) the further efficiency improvement of aptamer selection is highly needed and the improvement of the binding ability of selected aptamer to target analytes is also in urgent need. Also, the stability and repeatability of the constructed aptamer based methods should be widely validated and guaranteed. Meanwhile, given the selected aptamer probe, the aptamer based biosensor would hold great promise in the field of small molecules detection due to the difficulties in preparing corresponding antibodies. Therefore, following research of aptamer based biosensor in various fields, especially in food safety monitoring, should focus on the high efficiency and low-cost screening protocol for SELEX to find more available aptamer probes and the further optimization of stability of the developed aptamer based methods in practical applications. Anyway, in spite of many difficulties to be overcome, the aptamer based methods can be reached in various fields after continuous and intensive efforts.

Acknowledgments

This work is financially supported by the Huangshan Young Scholar Fund of Hefei University of Technology (407-037025), the National Natural Science Foundation of China with grant 31328009 and the NSF of Jiangsu Province (BK20130379, 13KJB550001), the Science and Technology Research Project of General Administration of Quality Supervision, Inspection and Quarantine of P. R. China (201210127, 201310135), the 12th Five Years Key Programs (2012BAK08B01-2, 2012BAK17B10, SS2012AA101001), Suzhou Science and Technology Committee Program (SS201335) and the Fundamental Research Funds for the Central Universities (2013HGCH0008, 2012HGCX0003).

G Liu acknowledges financial support from the National Cancer Institute (Grant number: R21CA137703) and the National Institute of General Medicine (NIGMS) (5P30 GM103332). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH.

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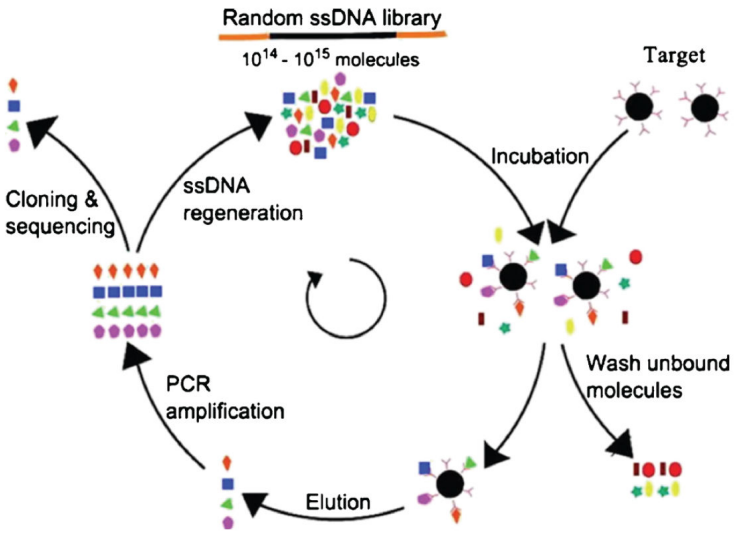

Fig. 1.
Schematic description of the process of SELEX [19]. Copyright 2012, ACS Publications

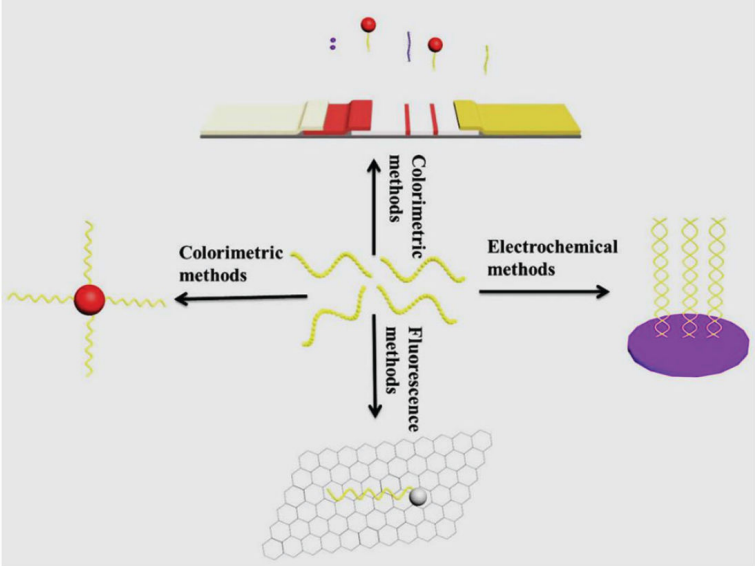

Fig. 2.
Application fields of aptamers in biosensors

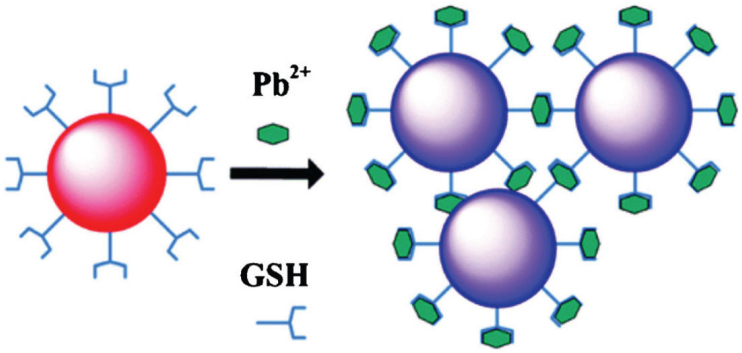

Fig. 3.

Strategy for colorimetric detection of Pb^{2+} by using GSH-GNPs [59]. Copyright 2010, ACS Publications

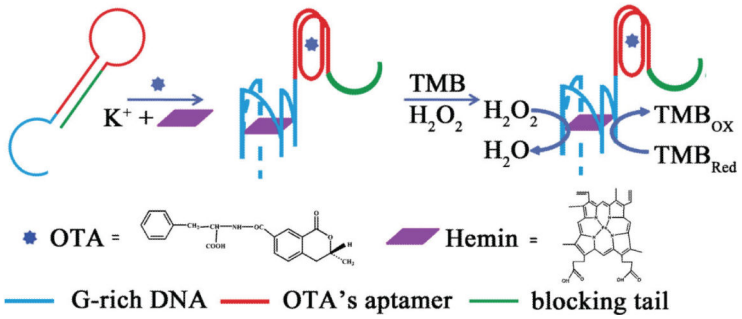

Fig. 4.

The principle of the bioassay for detection of OTA [63]. Copyright 2012, Elsevier

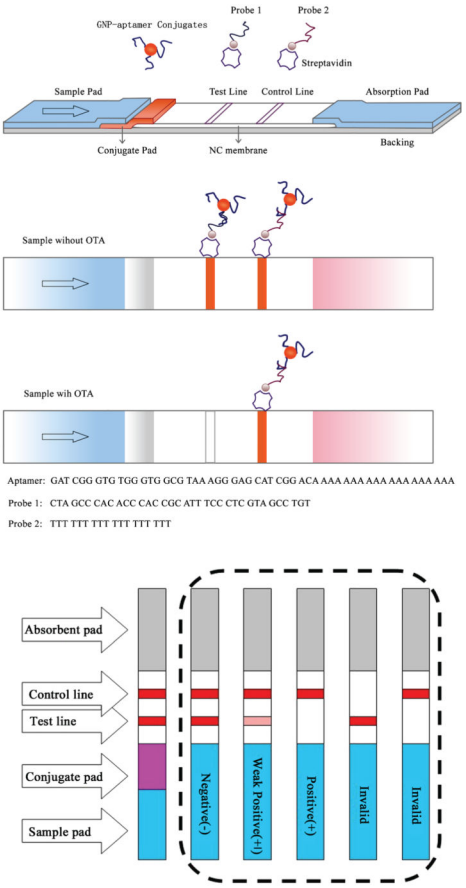

Fig. 5.

Schematic diagram of the principle for the detection of aptamer-based strip [65]. Copyright 2011, Elsevier

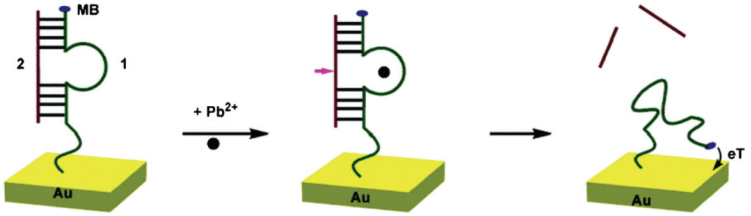

Fig. 6.

A schematic of the DNAzyme-based electrochemical sensor [70]. Copyright 2007, ACS Publications

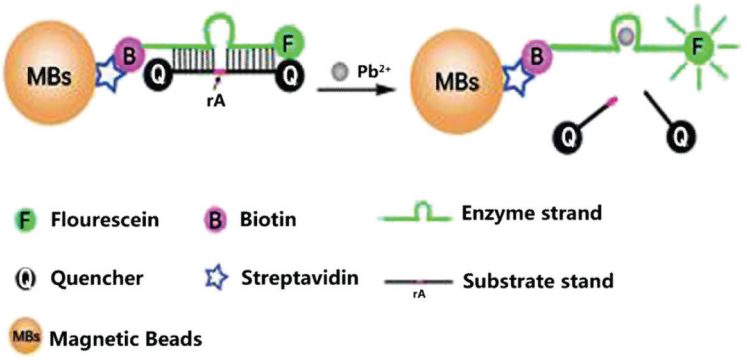

Fig. 7.

Schematic illustration of the DNAzyme-based Pb^{2+} sensor [76]. Copyright 2012, The Royal Society of Chemistry

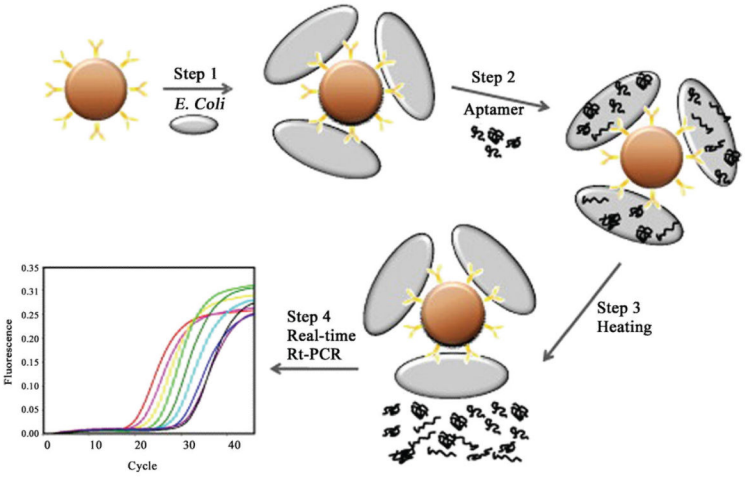

Fig. 8.

Scheme for the Escherichia coli detection in a combined approach of immunomagnetic separation, sandwich binding, and aptamer amplification [85]. Copyright 2009, Elsevier

Table 1

Summary of aptamers to hazards in food safety

Target	Target	Sequence of aptamer	Ref
Illegal additive	Bisphenol A	GGGCCGTTCGAACACGAGCATGCCGGTGGGTGGTCAGGTGGGATAGCGT	[34]
	17-β estradiol	TCCGCGAATTACACGCAGAGGGTAGCGGCTCTGCGCATTCAATTGCTG CGCGCTGAAGCGCGGAAGC	[35]
Bio-toxins	Ochratoxin A	GATCGGGTGTGGGTGGCGTAAAGGGAGCATCGGACA	[36]
	Botulinum Neuro toxin	GGGAGGAGGAGAGATGTGAACTTAUUCGGGCCCAGGAACCAACUAUAU AAAUGUCCCGAAUGCUUCGACGAGAAACTCTACACTGGACTGGCG	[37]
	Fumonisin B	AATCGCATTACCTTATACCAGCTTATTCAATTACGTCTGCACATACCAGCTT ATTCAATT	[38]
	AFB1	GTTGGGCACGTGTTGTCTCTCTGTGTCTCGTGCCCTTCGCTAGGCCCAC	[39]
	Ricin	CCGTAGGTTCGGGGCGGAGTGGTCCGGAAGGTGGCGTGG	[40]
Foodborne pathogen	Salmonellae enteric serovars	TTTGGTCCTTGTCTTATGTCCAGAATGCTATGGCGGCGTCACCCGACGGG GACTTGACATTATGACAGATTTCTCCTACTGGGATAGGTGGATTAT	[41]
	Campylobacter Jeijuni	TCATCCGTCACACCTGCTCT-N19-GGTGGTGTTGGCTCCCGTAT[a]	[42]
	Listeria monocytogenes	GGT TACTGA AGC ATATGT CCG GGG GAT TGC CAA GCCTTC CC	[43]
	Staphylococcus aureus	GGGCTGGCCAGATCAGACCCCGG ATGATCATCCTTGTGAGAACCA	[44]
	Lactobacillus acidophilus cells	TAGCCCTTCAACATAGTAATATCTCTGCATTCTGTGATG	[45]
Heavy metal	Uranyl ion	CTGCAGAATTCTAATACGACTCACTATAGGAAGAGATGGCGACATCTCTGC AGTCGGGTAGTTAAACCGACCTTCAGACATAGGCAGGCGTATATCTTGT GTCGGTAAGCTTGGCAC	[46]
	Mercury ion	TTCTTCTTTCTTCCCCCCTTGTTTGTTGTTT	[47]
	Lead ion	GGTTGGTGTGGTTGG	[48]
Durg residue	Tetracycline	CGTACGGAATTCGCTAGCCCCCCGGCAGGCCACGGCTTGGGTTGGTCC CACTGCGCGTGGATCCGAGCTCCACGTG	[49]
	Kanamycin B	CACCTAATACGACTCSCTATAGCGGATCCGAAGATGGGGGTTGAGGCTA AGCCGACCGTAAGTTGGGCCGTCTGGCTCGAACAAGCTTGC	[50]
	Malachite green	GGAUCCCGACUGGCGAGAGCCAGGUAACGAAUGGAUCC	[51]
	Anilinophenylacet-ic acid	ATACCAGCTTATTCAATTGGCAGTAGGTGTACATGCAAAGCCAGTGTGG GTCCCTGTGTAGATAGTAAGTGCAATCT	[52]
	Acetamiprid	CCTGCCACGCTCCGCAAGCTTTGTAATTTGTCTGCAGCGGTTCTTGATCG CTGACACCATATTATGAAGATAAGCTTGGCACCCGCATCGT	[53]
	Lividomycin	GGGAAUGGAUCCACAUCUACGAAUUCCCGUCAAGUCCGGUAAGGUGCC UG	[54]
		ACGUCUACUCCGACUUGACGAAGCUU	

[a] N_{19} refer to 19 random nucleotides

Table 2

Summary of hazards in food safety detection methods

Methods	Targets	LOD	References
Absorptiometric methods	Pb^{2+}	100 nM	[59]
	Hg^{2+}	0.5 μM	[60]
	Hg^{2+}	30 nM	[61]
	Hg^{2+}	100 nM	[62]
	Ochratoxin A	2.5 nM	[63]
	Ochratoxin A	20 nM	[64]
	Kanamycin	25 nM	[68]
	Melamine	0.98 ppb	[69]
Electrochemical based methods	Pb^{2+}	0.3 μM	[70]
	Pb^{2+}	0.1 pM	[73]
	Hg^{2+}	1 pM	[73]
	Ochratoxin A	30 ppt	[74]
	Ochratoxin A	0.065 ppt	[75]
	Tetracycline	10 nM	[72]
	17β-estradiol	10 nM	[35]
Fluorescent methods	Pb^{2+}	0.98 ppb	[76]
	Pb^{2+}	0.4 nM	[77]
	AFB1	0.11 ppb	[78]
	Ochratoxin A	18.7 nM	[80]
SPR	Pb^{2+}	1 pM	[82]
	Neomycin B	10 nM	[83]
rt-PCR	Escherichia coli	10 CFU mL^{-1}	[85]