Aptasensor Technologies Developed for Detection of Toxins

Ece Eksin, Gulsah Congur and Arzum Erdem

Abstract Aptamers are defined as new generation of nucleic acids which has recently presented the promising spesifications over to antibodies. They can be produced in vitro by **S**ystematic **E**volution of **L**igands by **EX**ponential Enrichment **(SELEX)**, and have the ability to recognize selectively and sensitively their targets; protein, toxin, drug or cell targets. Thus, they have a wide range of applications in different areas, such as, drug delivery, imaging and biosensing. Accordingly, an increasing number of studies related to aptamer based sensors "aptasensors" have been introduced in the literature. The recent studies on development of aptasensor technologies, which were applied for toxin detection, have been overviewed herein.

Keywords Aptamer · Aptasensors · Toxins · Optical aptasensors · Electrochemical aptasensors

1 Introduction

The rapid detection and monitoring of toxins in clinical fluids, environmental samples and foods require new approaches in order to expedite appropriate detection systems. Many toxins are secreted by bacteria during the course of infection and can be detected in low ng mL^{-1} quantities in urine or blood samples. Toxins in environmental samples can be introduced by industrial, agricultural, or military activity. Toxic compounds may also be found in environmental samples as a result of terrorist activity. Of particular concern for homeland defense are toxins that can be used as weapons; these include ricin, botulinum toxins, staphylococcal enterotoxin B (SEB), trichothecene mycotoxins, and saxitoxin [1]. Toxins also occur naturally in the food supplies. Mycotoxin contamination is a particular problem due to fungal infection of grains and peanuts and can still be present after

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food processing [2, 3]. While many cases of foodborne illnesses are caused by bacteria (e.g., salmonellosis, campylobacteriosis), a large number of illnesses are also caused by bacterial toxins, that have been secreted into the foodstuff during growth (e.g., Staphylococcus aureus enterotoxins, botulinum toxins) [4]. They also cause death in longterm. Due to their vital side effects, the advanced and faster detection protocols for toxins with better sensitivity and specifity has become an emerging necessity.

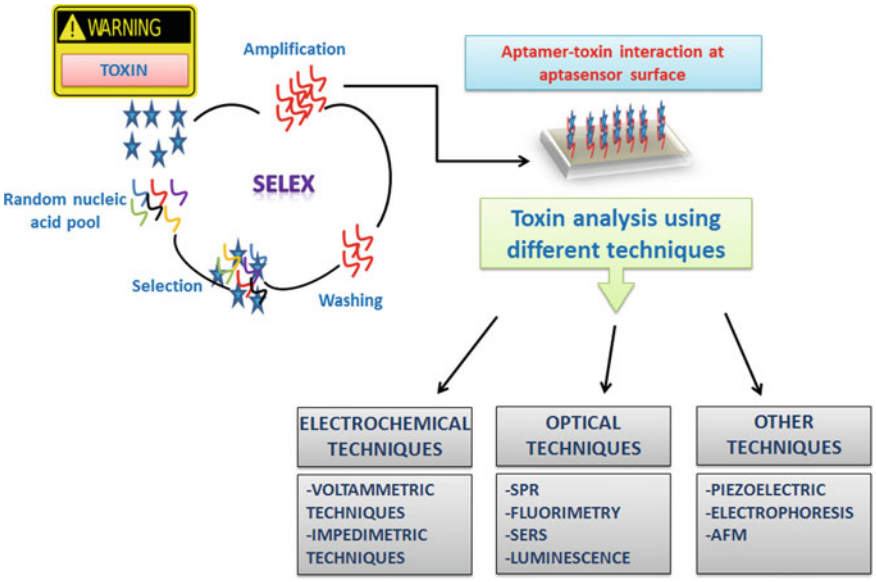
Aptamers are a class of new generation nucleic acids, which can recognize the target molecules specifically. Since their discovery in 1990 by Tuerk and Gold [5] they could be synthesized as single stranded DNA or RNA oligonucleotides using **S**ystematic **E**volution of **L**igands by **EX**ponential Enrichment (SELEX) method, which mimics the natural selection [5, 6] SELEX comprises tree fundamental steps; *(i) the* creation of a nucleic acid pool and the incubation with the target molecule, *(ii)* the generation of the specific bounds and separation of nonspecific bounds and finally *(iii)* the amplification of the bound molecules. Due to SELEX providing the design of the aptamer molecules, which have strong affinity to their targets, aptamers can be utilized to recognize a variety of (bio)molecules such as toxins [7] proteins [8–33] drugs [34–35] and even whole cells [36, 37]. Therefore, they have a great potential to apply for development of analysis systems toxins in the field of food [7, 34, 35, 38] medicine [8, 39]and environment [40, 41].

Biosensors are analytical devices, that are aimed to detect the analytes sensitively and selectively. Their structure allows to occur a specific response in the presence of the biological recognition element and the target molecule [10, 15, 18, 29–31]. Then, the response is converted into an electrical signal via a transducer. There are different types of transducers designed by using quartz cyrstral microscopy (QCM), surface plasmon resonance (SPR), optical or electrochemical techniques. Aptamers can be succesfully manipulated to develop biosensor systems and their combination is called as “aptasensors”. Aptamers are assessed in a wide range of biosensor designs due to their specifity against to analytes. Moreover, they promote the development more stable and robust platforms in comparison to antibodies which is a result of SELEX method. Consequently, there are many reports emphasizing the development of biosensors in combination with aptamer technology for detection of toxins [7, 42–62].

The recent studies on different aptasensor technologies, which were applied for detection of numerous toxins have been overviewed herein, and an aptasensor technology was simply represented in Scheme 1.

1.1 Electrochemical Aptasensors for Detection of Toxins

Toxins are small molecules produced from living organisms such as bacteria and fungus and have extremely serious effects on human health within very short time



Scheme 1 A representative aptasensor technologies developed for detection of numerous toxins

[42, 63, 64]. Their importance is about medical diagnosis, environmental monitoring, and food safety surveillance [42, 44, 63, 64]. Thus, monitoring of toxins via fast, reliable, sensitive and selective detection platforms has been gained attraction by researchers. In the meantime, aptamers were introduced in the field of development of biosensing platforms. One of them is electrochemical aptasensor technologies. Some approaches in the field of electrochemical aptasensors have been progressed for detection of toxins and given in Table 1 [35, 42–50, 64–70].

Ochratoxins are well-known by-products of numerous fungal species, which can contaminate not only foods, but also beverages including, coffee, beer, and wine. They are mainly produced in the *Aspergillus* and *Penicillium* genera [71]. Due to the fact that ochratoxin A (OTA) is known as the most toxic and has hepatotoxic, nephrotoxic, teratogenic and mutagenic effects onto a wide range of mammalian species [71–73], there are many electrochemical aptasensor applications in the literature to detect OTA [35, 65–67]. Zhang et al. [66] developed an electrochemical aptasensor by using gold electrode. They immobilized single stranded thiolated DNA aptamer labelled with biotin group onto the surface of gold electrode. The interaction of OTA and its DNA aptamer was then performed at the electrode surface and the interaction was determined in the presence of the resistance against TaqaI enzyme occurred after interaction process. Then, the enzymatic reaction between streptavidin-HRP and 3,3′,5,5′-tetramethylbenzidine sulfate (TMB) was monitored by using chronoamperometry technique.

Table 1 Some electrochemical aptasensors developed for detection of toxins

Type of toxin	Electrode	Method	Detection limit (DL)	Reference
ATX	AuE	CV, EIS	0.5 nM	[42]
BoNTA, RTA	AuE	SWV	0.4 ± 0.2 nM for BoNTA and 0.7 ± 0.5 nM for RTA	[43]
BoNTA	16-unit gold array	Amperometry	40 pg/mL	[44]
	rGO/AuE	CV, DPV, EIS	8.6 pg/mL	[64]
	AuE	CV, EIS	Not reported	[45]
AFB1	Dendrimer modified AuE	CV, EIS	0.40 ± 0.03 nM	[46]
AFB1	GCE	EIS	0.05 nM	[47]
AFM1	SPE	EIS	1.15 ng/L	[48]
	IDA	CV, SWV	1.98 ng/L	[49]
VerA	AuE	DPV	10 pg/mL	[50]
OTA	SPE	Amperometry	0.05 μg/L	[35]
	GCE	CV	0.03 ng/mL	[65]
	GCE	Chronoculometry	0.4 pg/mL	[66]
	AuE	CV, DPV, EIS	0.75 pM	[67]
TOA	SPE	CV	1 nM	[68]
BTX-2	Au electrode	CV, EIS	106 pg/mL	[69]
FB-1	GCE	EIS	2 pM	[70]

Abbreviations ***Toxins:*** *OTA* Ochratoxin A, *AFB1* Aflatoxin B1, *AFM1* Aflatoxin M1, *BoNTA* Botulinum neurotoxin, *BTX-2* Brevetoxin-2, *FB-1* Fumonisin B1, *SEB* staphylococcal enterotoxin B, *TOA* toxin A, *RTA* Ricin chain A, *VerA* Versicolorin A, ZEN: ***Type of electrodes:*** *AuE* Gold electrode, *rGO/AuE* reduced graphene oxide modified gold electrode, *GCE* Glassy carbon electrode, *SPE* Screen printed carbon electrode, *IDA* interdigitated electrode array. ***Detection methods:*** *CV* Cyclic voltammetry, *SWV* square wave voltammetry, *DPV* differential pulse voltammetry, *EIS* electrochemical impedance spectroscopy

In the study reported by Rhouati et al. [35], a fully automated flow electrochemical aptasensor based on the magnetic beads (MBs) was introduced and accordingly, direct and indirect competitive electrochemical assays were developed to monitor OTA. For fabrication of this direct assay, carboxylated aptamer modified MBs were immobilized onto the surface of screen printed carbon electrode (SPCE) placed in a flow cell. After the immobilization of avidin-ALP onto the surface of the electrode, the enzymatic reaction in the presence of 1-naphthyl phosphate was occurred and the oxidation of the electro-active product 1-naphtol phosphate to 1-iminoquinone was detected by using amperometry. For fabrication of indirect assay, OTA modified MBs were immobilized onto the surface of SPCE. The free OTA molecules and the immobilized OTA molecules were competed in the solution for binding of biotinylated DNA aptamer. The avidin-ALP was then conjugated and the enzymatic reaction was utilized. A lower limit of detection

(0.05 μg/L) was obtained with the indirect flow-based aptasensor both of the electrochemical assays were tested in the presence of buffer, or beer samples.

Aflatoxins are known to be carcinogen and highly toxic secondary metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus* [46–48]. FDA limited the level of aflatoxins in nuts, seed and legumes. The monitoring and the detection of aflatoxin at low levels has become attractive in the food safety area. Therefore, some studies were reported for development of electrochemical aptasensors for detection of aflatoxins [46–49]. Nguyen et al. [49] fabricated an electrochemical aptasensor platform for monitoring of AFM1. They used Fe_3O_4 incorporated polyaniline (Fe_3O_4/PANI) film modified interdigitated electrode (IDE) as electrochemical aptasensor platform. They found the detection limit as 1.98 ng/L. In another study, an impedimetric aptasensor onto SPE surface was developed and used for detection of AFM1 [48]. The detection of AFM1 was achieved based on the changes at the charge transfer resistance (R_{ct}) even in milk samples.

Fumonisin B1 (FB-1) is primarily produced by *Fusarium moniliforme* and the most abundant and important fumonisin [74]. It has been found in maize, maize products animal feeds [75]. FB1 threats both animal and human health [76, 77]. An impedimetric aptasensor was developed by Chen and coworkers [70] for recognition of FB-1. GCE surface was modified gold nanoparticles (AuNP) and the interaction of DNA aptamer and FB-1 was investigated based on the changes at the R_{ct} value. The selectivity of the aptasensor was then tested against other toxins.

1.2 Optical Aptasensors for Detection of Toxins

Aptamers have been used as bio-probes in optical sensors based primarily on the incorporation of a fluorophore or a nanoparticle. In the case of fluorescence detection, the simplest format is to label the aptamers with both a quencher and a fluorophore. Additionally, many nano-materials, including QDs, AuNPs, CNTs, graphene oxide (GO), polymer nanobelts, and coordination polymers, have been investigated for their fluorescence-quenching effect instead of using a more traditionally quencher [78–84]. Some optical aptasensors developed for detection of toxins were summarized in Table 2.

AuNPs or several polymers that cause color changes, can be applied as novel reagents for the optical detection technique called colorimetry. The highly negatively-charged ssDNA (complementary strand of the aptamer), which is separated from the aptamer by interaction between the aptamer and the target, is stabilized against aggregation, and a color change occurs in conjunction with this phenomenon [85].

The light chain of BoNT/A (LCA) was utilized as target molecules in SELEX process. Overall, Chang et al. [86] identified three RNA aptamer species which have high binding affinity, specificity and strong inhibition activity. They showed that the endopeptidase activity was effectively inhibited by docking of aptamer to

Table 2 Some optical aptasensors developed for detection of toxins

Type of toxin	Type of substrate	Method	Detection limit (DL)	Reference
OTA	Au chip	SPR	0.005 ng/mL	[54]
LPS	AuE	SPR	–	[55]
OTA	–	Fluorescence	21.8 nM	[56]
OTA FB-1	–	Fluorescence	0.02 ng/mL 0.01 ng/mL	[57]
Abrin	–	Luminescence	1 mM	[58]
BoNT	–	Spectroflorimetry	1 ng/mL	[59]
AFB1	–	Chemilumiescence	0.11 ng/mL	[7]
Ricin	SERS substrate	SERS	10 ng/mL	[60]
Ricin	SERS substrate	SERS	25 ng/mL	[61]
Ricin B	Silicon substrate	SERS	0.32 fM	[62]

Abbreviations ***Toxins:*** *OTA* Ochratoxin A, *AFB1* Aflatoxin B1, *BoNT* Botulinum neurotoxin, *FB-1* Fumonisin B1, *LPS* Lipopolysaccharide. ***Electrodes:*** *AuE* Gold electrode. **Method:** *SPR* Surface plasmon resonance, *SERS* surface-enhanced Raman scattering

BoNT/A (LCA). Their study was the first to confirm that the aptamers for the light chain BoNT/A (LCA) could be used as therapeutic reagents against the deadly botulism [86].

1.3 Other Techniques Developed for Detection of Toxins Using Aptamer Technologies

There are some reports in the literature which can be classified as aptasensors. Nanogold modified piezoresistive microcantilevers (PZR) were used for monitoring of S*taphylococcus enterotoxin* B (SEB) which is small monomeric protein and a pathogen with high thermal and proteolytic stability [87]. PZR sensor surface was modified with DNA aptamer, then the interaction of SEB and its DNA aptamer was investigated even in milk samples.

Ricin is a plant lectin from the castor bean plant *Ricin communis* [51]. It consists of two chains, an A chain and B chain linked by a single disulfide bond and the A chain is toxic to cells [52]. Its production is relatively easy and it is a potential threat as a terrorist weapon. Capillary electrophoresis based aptasensor was reported by Haes et al. [52] for monitoring of ricin A chain. The interaction of ricin and DNA aptamer was performed in capillary surface. Detection of ricin could be achieved in nuclease-contaminated sample matrixes. In another study, atomic force microscopy (AFM) based aptasensor was developed for monitoring of ricin [53]. DNA aptamer and ricin interaction was performed at the surface of Au(111) and ricin binding sites to aptamer was predicted.

2 Conclusion

Aptamers have been utilized in biosensor area since their discovery by Tuerk and Gold [5] due to their stability against physical conditions such as ionic strength, temperature and pH and production cost. They have been alternative biorecognition elements for antibodies even their discovery is relatively new [88, 89]. Aptamers synthesized and isolated by SELEX procedure can spesifically recognize their targets even in complex matrix due to characteristic structure generated during SELEX procedure. They have been used for recognition of proteins [21, 29, 30, 32, 33, 63, 90–92], drugs [93–95] and also toxins [7, 42–62, 64, 96, 97] in combination with different detection techniques such as optic, colorimetric, electrochemical, or piezoelectric techniques. Aptasensors developed for toxin analysis have offered the advanced assays for sensitive, selective, fast, reliable and cost-effective monitoring of numerous toxins as well as their application into the real samples such as food matrices, or biological fluids.

In another aspect, aptasensors can be miniaturized and adaptable for chip technologies for development of aptasensors based on point of care systems which are portable, compatible and having an easy-to-use design. Thus, their application to the environmental or food samples such as water, milk, nuts etc. could be performed and toxins could be sensitively and selectively analyzed with on-line measurements via aptamer based chip technologies in a short time.

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