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Aptamer-based environmental biosensors for small molecule contaminants

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Aptasensors are promising biosensors, which take advantage of using aptamers as a recognition element. The combination of the excellent characteristics of aptamers and the leading detection platform techniques, such as optical, electrochemical with nanomaterial-integrated, or mass-sensitive techniques with high sensitivity and specificity draws a promising view for the application of the aptasensors for the detection of harmful small toxic chemicals and real-time monitoring in the environments. In spite of attraction of aptasensors, application of them is limited to the complex environment due to the facts that how the immobilization of aptamers onto the surface affects the functions of aptamers and their structures for the detection of environmental contaminants are not clearly known. This review examines the most recent update on the selection of aptamers for small molecules, the development and application of aptasensors in the detection of small molecule contaminants in environment. Additionally, their applications to the real samples as environmental monitoring reported in the publications also are reviewed.

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Introduction

Nowadays, monitoring requirements of environmental contaminants are ever-increasing, since the chances of exposure by toxic chemicals that can affect human and animal life are huge. Some contaminants cause mild effects even after long term exposure, however, some others can have deadly effects and lead to global disasters. Although there have been a lot of efforts in developing techniques for monitoring various environmental toxic molecules, there is still a great need in particular for portable, field deployable, and highly robust technologies.

The pollutants that need to be monitored in the environment can be broadly divided into four classes: toxins, pesticides, environmentally polluting hormones and persistent organic toxic chemicals (POTC), and pharmaceuticals and personal care products (PPCPs). Since most of those are small molecules less than 1000 Da, they are non-immunogenic and antibodies are inappropriate as recognizing receptors. In other cases, it is too tedious and complicated to develop the antibodies by binding the small targets to a certain protein. Moreover, due to expensive production costs for the synthesis of antibodies and their instability upon exposure to environmental conditions, alternatives are needed. Aptamers as recognizing receptors into environmental monitoring systems have attracted attention and their applications have been continuously studied.

Aptamer-based environmental biosensors, so called aptasensors, have been developed especially for the detection of harmful toxic agents in the environment. Aptamers are short single-stranded nucleic acids obtained in a process, which is called SELEX (for Systematic Evolution of Ligands by EXponential enrichment). Applying aptamers to biosensors can have many advantages. They show high flexibility and stability, are cheap to produce, and easy to modify. These are interesting properties for real sample applications. Aptamers can be developed by *in vitro* screening of the various types of targets, ranging from small molecules to whole cells, thus making it possible to develop a wide range of aptasensors [1–4]. However, there are a few drawbacks in the conventional SELEX procedure using solid supporting materials (SSMT-SELEX): 1) upon immobilization of the target molecule, around one of third of target surface is lost or inaccessible; 2) immobilization steps become very complicated or unsuitable for small molecular targets, in particular when molecules do not have any functional groups or are difficult to conjugate; 3) Some properties of the targets may change upon modification and thus affect the affinity of the aptamer to bind the original free target molecule. To overcome these issues, new SELEX procedures have been developed recently, such as structure-switching SELEX and Capture-SELEX [5,6]. The newer methods, however, still may lose some of the high affinity existing aptamers in the procedure, resulting in an enrichment of selected aptamers with low affinities. In addition, aptamer refolding relies mostly on their random region, especially for small molecule targets, which also results in selection

of aptamers with low affinities. In response to this, Gu and co-workers have developed an immobilization-free aptamer screening method based on graphene oxide (GO) assistance (GO-SELEX) [7••]. In this procedure, instead of immobilizing the targets on a rigid surface, the random DNA pool is adsorbed on a GO sheet via the π–π stacking interactions with the surface. The key advantage of GO-SELEX is that, especially, small molecular targets are placed without modification. In addition, aptamer selection is independent of the target's size and molecular weight, because of target-induced aptamer detachment from the GO surface [8–11]. A recent demonstration included Multi-GO-SELEX to screen for flexible high-affinity aptamers for multiple pesticides [12•].

Aptamer-based environmental biosensors for small molecule contaminants

Among many different signal transducing platforms available for aptasensors, some limitations exist for implementing every platform technology, mainly due to the size of the target molecules. In other words, some of the platform technologies based on the mass of the targets or the multiple binding capabilities of the targets are hardly applied to the environmental monitoring. So, in this review, it is highlighted how these limitations caused from the intrinsic nature of the environmental contaminants, such as its small molecular weight or non-modifiable targets, are appropriately dealt or overcome by introducing nanotechnology or complicated multiple steps, even though some of the methodologies are not feasible in field.

Optical aptasensors

Fluorescence detection is widely employed for aptamer–ligand interactions, due to its convenience of labeling the aptamers with fluorescent dyes, the choice of many different fluorophores and quenchers, and the inherent capability for real-time detection. Several main strategies have been developed for converting aptamer–ligand binding into fluorescent signals, such as molecular beacons, duplex structure with complementary sequences, and competitive laser-based flow assays (Figure 1). The most famous and widely used fluorescence method to detect ligand-binding is by molecular structure-switching [13–20]. Turn-off style lateral flow assays (LFA) for aptasensors have been developed particularly for small molecular targets, which are based on the target-induced displacement of aptamers [21•]. As an example, aflatoxin-B binding aptamers were modified by biotin and their complementary DNA sequences were labeled with Cy5 as a signal probe.

Another principle uses salt-dependent aggregation of gold nanoparticles (AuNPs), of a size of 10–50 nm, leading from a color change of wine red to purple and size-dependent surface plasmon absorbance peak shifts. The aptamers are placed on the surface of AuNPs and prevent aggregation via electrostatic repulsion. In presence of the ligand, the aptamers detach out from the AuNP surface to bind their own targets, and this induces the nanoparticle aggregation [22]. Based on this principle, a number of colorimetric aptasensors were developed for different environmental toxic chemicals, such as for Bisphenol A [23], Ochratoxin A [24], and pesticides [10,12•]. Since the AuNP-based colorimetric aptasensors have relatively low compound detection sensitivities, horseradish peroxidase (HRP) mimicking DNAzyme has been introduced, which enhances the detection of the aptasensor [25•]. As example, a competitive assay using an aflatoxin B1 (AFB1)-ovalbumin coated 96 well plate was developed with aptamers specific for AFB1, which generated chemi-luminescence from a hemin/G-quadruplex horseradish peroxidase-mimicking DNAzyme (HRP-DNAzyme) that was linked to the AFB1-specific aptamer. The advantage of using a DNAzyme forming a hemin-G-quadruplex complex, is that it shows higher catalytic activity compared to hemin itself.

Electrochemical aptasensors

Electrochemical detection of small molecule binding to aptasensors has recently been developed on different platforms (Figure 2). Among them, label-free electrochemical impedance spectroscopy (EIS) has appeared as a promising strategy. EIS is not only a powerful method to characterize biomolecule-functionalized substrates, but also a sensitive technique to monitor aptamer–ligand binding occurring on the electrode surface. More importantly, EIS is nondestructive, which makes it highly attractive for aptamer-based small molecule detection [26,27]. Demonstrating one of the first electrochemical aptasensors, Kim *et al.* reported detection of 17β-estradiol [28]. The 17β-estradiol was detectable up to 0.1 nM, and the linear range of this biosensor was from 0.01 to 1 nM. Another label-free competitive electrochemical aptasensor was described for Brevetoxins (BTXs) [29], which was based on the competition between the BTX-beads and BTX-HRP conjugate. The limit of detection in this aptasensor for BTXs was calculated at 106 pg/ml. Elshafey *et al.* reported a newly selected aptamer embedded in an EIS aptasensor for the detection of anatoxin-a (ATX), the smallest potent neurotoxin [30]. This aptasensor exhibited an excellent detection limit (0.5 nM), and a linear range from 1 nM to 100 nM.

More recently, electrochemical aptasensors integrated with nanomaterials have shown great potential for detection of small molecules. Fan *et al.* reported an ultrasensitive photoelectrochemical (PEC) sensing platform for detection of 17β-estradiol (E2) based on TiO_2 nanotube arrays modified with CdSe quantum dot nanoparticles [31••]. The CdSe nanoparticles were electrodeposited on the inner and outer surface of the TiO_2 nanotubes, and the E2 aptamer was immobilized onto the CdSe nanoparticles attached to the TiO_2 nanotubes (Figure 3a).