Chapter

A New Approach for Detection of Aflatoxin B1

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Abstract

Aflatoxin B_1 (AFB_1) is harmful to human health, mainly resulting from its toxic effects on the liver. AFB_1 can lead to liver cell necrosis, hemorrhage, fibrosis, cirrhosis, etc. Acute AFB_1 exposure at high levels can lead to hepatitis, whereas chronic exposure can result in liver cancer. In the past decades, a series of methods and techniques for detecting AFB_1, including enzyme-linked immunosorbent assay (ELISA), high-performance liquid chromatography (HPLC), and thin-layer chromatography (TLC), have been developed. This study reviewed the detection methods of AFB_1 and the corresponding utilization and summarizes all methods for evaluating the toxification of AFB_1.

Keywords: aflatoxin B_1, purification, detection, aptasensor, biosensor, reduced graphene nanosheets

1. Introduction

Aflatoxin B_1 (AFB_1) is mainly a metabolite produced by *Aspergillus flavus*. AFB_1 poses a threat to human health due to its three huge toxicities [1]. The toxic effects are as follows [2]. First, it is genetically toxic and can cause DNA damage. Second, aflatoxin shows strong hepatophilic properties when it enters the human body and can cause liver cell necrosis, hemorrhage, fibrosis, and cirrhosis. Finally, aflatoxin has high toxicity and strong carcinogenicity. The data show that its toxicity is 10 times that of potassium cyanide and 68 times that of arsenic. The carcinogenic force is 70 times that of the known carcinogen dimethyl nitrosamines and 900 times that of butter yellow (methyl azobenzene) [3]. The carcinogenic pathway is mainly activated by cytochrome p450 (CYP) monooxygenase system, and AFB_1 is metabolized by CYP1A2 and CYP3A4 to produce epoxy compounds, including active epoxy resins (aflatoxin-8,9-epoxy, AFBO), which generate mutagenic aflatoxin-n7-guanine adduct (AFB1-N7-gua) through interaction with DNA and cause DNA damage to varying degrees [4]. Since AFB_1 is toxic to the human body, it is necessary to monitor the content of AFB_1 in food. However, AFB_1 pollution still exists in a small number of remote areas due to poor living standards and quality. Considering the feasibility and economic feasibility of AFB_1 detection technology, the government needs to add feasible, fast, and accurate new technical schemes for supervision [5]. Therefore, it is of great significance to study the new progress in the detection of AFB_1 in food.

Detection of AFB_1 is divided into two processes, including purification of AFB_1 and quantitative analysis of AFB_1. Purification methods of AFB_1 mainly include liquid–liquid extraction, dispersive liquid–liquid microextraction, solid-phase

extraction, molecularly imprinted polymer, immunoaffinity column, etc. [6]. The quantitative analysis methods of AFB_1 mainly include enzyme-linked immunosorbent assay (ELISA), high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC), etc. These methods are tedious, time-consuming, and expensive; moreover, the sample processing is complex and requires professional operation, which is not suitable for the rapid and effective detection and analysis of AFB_1. Therefore finding a fast and sensitive method has important application value.

With the rapid development of science and technology, scientists have been concerned about inventing a fast and sensitive method to detect AFB_1. AFB1 detection based on aptamer AFB_1 sensor is the most widely used detection technology. The aptamer is a single-stranded nucleic acid or peptide molecule; it has a unique secondary structure and can specifically bind to the target, like proteins, drugs, and other biomolecules [7]. Aptamer-based biosensors (aptasensors) have been widely used owing to high sensitivity, selectivity, accuracy, fast response, and low cost [8–10]. Fluorescent-based optical biosensors are the most commonly used method. Combining fluorescent pigment molecules with fluorescent aptasensors leads to the generation of light in the process of biological recognition interaction, so as to achieve the detection of target molecules [11]. In addition, nanomaterials have been widely used in biomolecular detection, such as graphitic carbon nitride nanosheets (g-C3N4 NSs) [12] and reduced graphene nanosheets (rGO) [13].

2. Purification of aflatoxins

The purification is the key step in the detection of the level of AFB_1; traditional methods of AFB_1 mainly include liquid–liquid extraction (LEE), dispersive liquid–liquid microextraction, solid-phase extraction (SPE), molecularly imprinted polymer, immunoaffinity column, etc.; most of these are time-consuming and expensive [14]. Encouragingly, Xie J et al. [6] provide the first report of a broad-spectrum specific mAb-modified reduced graphene nanosheets (rGO) film that can be designed to extract and purify AFB_1, AFB_2, AFG_1, AFG_2, AFM_1, and AFM_2 in rabbit serum. This method is suitable for analysis of different types of analyses from different samples. Compared with the traditional method, this method has the advantages of high selectivity, simplicity, low sample consumption, and the use of a small amount of organic solvent, especially extraction of ultra-trace levels of AFs.

However, in the process of extracting AFB_1, the complexity of food components, especially fat, causes some interference to AFB_1. In addition, AFB_1 is lipophilic; it is difficult to extract AFB_1 from soybean and vegetable oil [14, 15]. The purification is not strong enough, the AFB_1 in vegetable oil cannot be completely removed, and lower lever of AFB_1 will also lead to human liver damage [1, 16]. Xi Yua et al. analyzed trace amounts of AFB_1 in vegetable oils by combining LTC and immuno-magnetic solid-phase extraction (IMSPE) with fluorescence spectroscopy (FL) detection. This process removed fat interference in vegetable oil samples. Subsequently, IMSPE enhances the selectivity and efficiency of extraction through specific antibody–antigen binding. The advantage of this method is that the combined application of traditional LTC and modern IMSPE improves the sensitivity and selectivity of extraction process and meanwhile reduces the time and cost.

3. Application of aptamer

Nucleic acid aptamers are single-stranded oligonucleotides screened in vitro by systematic evolution of ligands by exponential enrichment (SELEX), which are

widely concerned as a new biometrics. SELEX technology can be used to screen the combination of the target molecule specific adaptor and target specific [17]. Thus the aptamer has the characteristics of simple preparation, strong specificity, good stability, and a very wide range of target substances, including analysis and detection, biochemistry, food safety [18], clinical medicine [19], and other fields [20]. According to the design principles in different fields, adaptors can be converted into different signals. The commonly used ligand biosensors include fluorescence adaptor sensor, colorimetric adaptor sensor, electrochemical adaptor sensor, etc. In recent years, the aptamer has been applied to the detection of AFB_1, which has greatly improved the detection efficiency and sensitivity of AFB_1 in the field of aflatoxin sensor construction; according to various researches at home and abroad, electrochemical biosensors have been constructed with antibody, enzyme and nucleic acid aptamer as recognition elements; and enzyme catalysis technology, DNA self-assembly technology, ionic liquid, nano materials, conductive polymer have been used to metal compounds, etc. for the detection of aflatoxin [21–23].

4. Fluorometric aptamer

Ye et al. [24] developed a low-cost, high-sensitivity fluorescence polarization (FP) assay by using GO-based fitness biosensors to detect AFB_1. Fluorescein amidite (FAM) labeled the aptamers fitness combines with the surface of GO to form the aptamer/GO macromolecular complex. In the presence of AFB_1, the opposite dissociates from the GO surface and binds to AFB_1 specifically to form the aptamer/AFB_1 complex. As a result, large changes in the molecular weight of the aptamer were observed before and after the combination, leading to significant changes in the fluorescence polarization (FP) value. The lowest detection limit (LOD) of this method was 0.05 nM.

Li et al. [25] use a fluorometric aptamer-based method to detect the level of aflatoxin B1 (AFB_1). Their assay aims to develop a simple and sensitive label-free fluorescence aptasensor to monitor and control AFB_1 in foodstuffs quickly and accurately. In their experiment, the AFB_1 aptamer with the fluorescent dye thioflavin T (ThT) forms a AFB_1 aptamer/ThT G-quadruplex complex in the absence of AFB_1, increasing the fluorescence intensity of ThT. While the AFB_1 aptamer with AFB_1 forms a AFB_1 aptamer/AFB_1 complex in the presence of AFB_1, causing the fluorescence intensity to decrease, the levels of AFB_1 were directly correlated to fluorescence intensity. The general experimental procedures are as follows: first of all, the samples were preprocessed; then, the experimental conditions were optimized, including the optimum ratio of AFB_1 aptamer: ThT, the concentration of KCL and the reaction time (20 min); lastly, using a LUMINA Fluorescence Spectrometer, the fluorescence intensity at excitation/emission wavelengths of 440 nm/487 nm was tested. In this case, the results were in good agreement with those obtained from commercial ELISA kits; the advantages of this method are simpler and more convenient—no label, low cost, and higher efficiency and specificity. The more evidence [8] has proven that this fluorometric aptamer-based method has great practical applications in food industry; not only does it detect AFB_1 and ochratoxin A, but it is more likely to spread to other toxins.

Xia et al. [26] designed a dual-terminal proximity structured aptamer probe; the main purpose of this design is to construct an enzyme-free, ultrafast, single-tube, homogeneous AFB 1 analysis method. This aptamer probe can quickly respond to AFB_1, and the detection process can be completed within 1 min, which is one of the fastest detection methods for AFB_1. Aptamer probe is the design to dual-terminal proximity structures, which allows the binding of one molecule to illuminate the

fluorophores of two molecules and achieve enzyme-free amplification and significantly improve the signal-to-background ratio and sensitivity of AFB_1 detection.

Lu et al. [27] discovered another interesting fluorescence method. Their experiments reported a target-driven switch-on fluorescence aptasensor for monitoring AFB_1 determination by employing the fluorescence resonance energy transfer (FRET) between the CdZnTe quantum dots (QDs) and Au nanoparticle (AuNP) pair. AuNPs is considered to be one of the most widely used metal NPs. It can promote electron transfer and act as a tiny conduction center. The crucial design of this switch is that the AuNP acceptors were bioconjugated with the thiol group-modified complementary DNA (cDNA) of aptamer. In this case, as the CdZnTe QDs (energy donor) approaches AuNPs(energy acceptor), FRET is produced, leading to the subsequent fluorescence disappearance of CdZnTe QDs, while AFB1 specifically binds to the aptamer, and aptamer breaks away from AuNPs. Thus, CdZnTe QDs separates from AuNPs, leading to the subsequent fluorescence recovery of CdZnTe QDs. This aptasensor is simple in design and has the advantages of wide linear range, low LOD, high sensitivity, and selectivity.

Wang et al. [28] synthesized a novel fluorescent nitrogen-doped carbon quantum dot (N, C-dots) and combined it with the aptamer/AuNP complex for detection of AFB_1. Initially, they synthesized a positively charged fluorescent N, C-dots by hydrothermal treatment of trypsin, synthesized AuNP by a typical citrate reduction method, and attached a thiol-labeled oligonucleotide (AFB_1 aptamer) to AuNP. N, C-dots/aptamer/AuNP nanocomposite is formed on the surface. N, C-dots are mainly used as a quencher for the construction of aptamer sensors. When AFB_1 is absent, N, C-dots bind to aptamer/AuNPs by electrostatic interaction, and the fluorescence of N, C-dots is quenched by AuNPs. When AFB_1 is present, the aptamer binds to AFB_1, N, C-dots are released, and its fluorescent signal is restored. Therefore, by measuring the fluorescent signal of N, C-dots, the concentration of AFB_1 can be obtained. The detection system is extremely sensitive with a detection limit of 5 pg/mL (16 pM).

Beheshti-Marnani et al. [13] developed aptasensor assembled with assisting reduced graphene oxide nanosheets as the signal amplifier was fabricated and applied for detecting ultralow levels of AFB_1 through a nanobiology interaction system. The detection principle and procedures are different from fluorescence method; the steps are as follows: (1) synthesis of reduced graphene nanosheets (rGO), (2) fabrication of the AFB_1 aptasensor, (3) immobilizing AFB_1 binding ssDNA aptamer on the surface of electrode, and (4) cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) characterized by the modified glassy carbon electrodes. Differential pulse voltammetry (DPV) was used to quantitatively analyze aflatoxin B_1 in practical samples. This new technology is characterized by its simplicity, low cost, and sensitive label-free, in particular, the ability to detect very small quantities of aflatoxin B_1 with a considerable low limit of detection (LOD = 0.07 nM) and good repeatability (RSD = 2.9) and stability.

Wang et al. [29] report a versatile ratiometric fluorescence platform for multiple detection of various targets based on the conjugation of single-stranded DNA (ssDNA) with protonated graphitic carbon nitride nanosheets (Pg-C3N4NSs). This method is also feasible for AFB_1; the principle is that Pg-C3N4NSs promotes oxidation of substrate o-phenylenediamine (OPD) by binding to ssDNA in the presence of H_2O_2. Subsequently, the fluorescence signal at 564 nm of the oxidation product 2,3-diaminophenazine (DAP) was collected and concurrently quenches the intrinsic fluorescence of conjugates ssDNA/Pg-C3N4 NSs at 443 nm upon excitation at 370 nm. Lastly, the transformation of fluorescence was used for ratiometric fluorescence-based analytical. This method applies to for multiplex detection of various targets.

5. Electrochemical aptamer

Wu et al. [30] proposed a simple electrochemical body sensor, and they take advantage of host-guest identification between ferrocene and β-cyclodextrin (β-CD) to detect AFB_1. Despite the long-time consumption and complexity involved in the preparation process of the pβ-CD/AuNPs/GC electrode and AFB1-sensitive dsDNA, they demonstrated the selectivity, stability, and reproducibility of the electrochemical aptasensor in the detection of AFB_1; there is no significant difference in stability between 1 day and 15 days, that is to say, electrochemical aptasensor has good stability.

Abnous et al. [31] built an electrochemical biosensor for accurate detection of AFB_1. AFB_1 is based on aptamer to form a π-shape complementary strand of aptamer (CSs) complex on the surface of electrode and exonuclease I (Exo I). The purpose of π-shape design is to greatly increase the sensitivity of aptamer. In the absence of AFB_1, the PI configuration of the gold electrode surface remains intact, and a double potential barrier is formed on the electrode surface, limiting the contact of $[Fe(CN)_6]^{3-}/^{4-}$ with the electrode surface, and only weak electrochemical signals are measured. When AFB_1 exists,π-shape structure was removed, and a strong current was recorded after the addition of Exo I. Under the optimum conditions, the concentration range of AFB_1 can be detected in the range of 7–500 pg/mL and a limit of detection (LOD) of 2 pg/mL.

Xia et al. [32] designed a new split-type photoelectrochemical (PEC) immunosensing platform for sensitive detection of AFB_1, combined with the etching reaction triggered by the enzymatic hydrolysis of cobalt oxyhydroxide (CoOOH) at the functional interface of cadmium sulfide (CdS) nanoparticles. The concentration of CdS nanoparticles has a great influence on the analytical properties of PEC biosensor. Excessive CdS may induce high background signal, while low concentration produces weak photocurrent response. In their experiment, the optimum concentration of CdS nanoparticles was 0.8 mg/mL^{-1}, and the entire time of the method is within 1.5 h for each sample. Under optimal conditions, the detection limit of this method is 2.6 pg/mL^{-1}, and the accuracy of this method (expressed in RSD) is ±8.6%.

6. Aptamers with chemiluminescence immunoassay

Li et al. [33] developed an aptamer structure switch experiment with horseradish peroxidase (HRP) labeling for sensitive absorbance and chemiluminescence detection of small molecules. Differently from competitive enzyme-linked immunosorbent assay (ELISA), they fixed the cDNA of the aptamer to the surface of the microporous plate.

7. Others

Zhao et al. [34] developed a novel nano-antibody and magnetic beads-based directed competitive ELISA (MB-dcELISA) based on both recombinant antibody and its mimotope for AFB1 detection. The 50% inhibition concentration and detection limit of MB-dcELISA were 0.75 and 0.13 ng/mL, respectively, and the linear range was 0.24–2.21 ng/mL.

Zhang et al. [35] discovered a novel anti-AFB_1 monoclonal antibody in order to establish a sensitive immunoassay for AFB_1, and a novel CdTe/CdS/ZnS quantum dot fluorescence probe was synthesized by binding to the surface of CdTe/CdS/ZnS

quantum dots. CdTe/CdS/ZnS quantum dot is a kind of semiconductor nanomaterial, which has strong photostability and fluorescence efficiency and has longer fluorescence time. Compared with the traditional ELISA method, fluorescence immunoassays (FLISA) can be used to measure AFB_1 in grain samples in a wide range of linearity. In addition, CdTe/CdS/ZnS quantum dot fluorescence assay has lower toxicity, high stability, and excellent fluorescence properties.

Based on the competitive response of AFB1 and cy5 modified DNA complementary strands to aptamers, Shim et al. [36] first developed a dipstick assay for AFB1 sensing. This sensor has a minimum detection limit of about 0.1 ng/mL for AFB_1, indicating good potential for practical applications. The whole determination process can be completed in 30 min. Moreover, the dipstick assay is consistent with the ELISA assay results.

8. Discussion

We mainly report on the new detection techniques of aflatoxin in recent years. It mainly includes fluorescence detection, electrochemical detection, immunological detection, and so on. Fluorescence detection method is more and more popular in the industry because of its high sensitivity and high specificity. Electrochemical detection is a powerful analytical technology. Due to its simple operation and low price, it has been widely used in environmental monitoring and food safety [30]. According to the different detection principles, it can be divided into electrochemical enzyme sensor, electrochemical immunosensor, and electrochemical aptamer sensor in the determination of aflatoxin in food. ELISA has been widely used in the determination of AFB_1 in recent years. The method has high sensitivity and selectivity, but the reaction time is long and the operation process is complex. In addition, antibodies and enzymes are easily denatured during storage, which severely limits their practical application.

More and more scholars are committed to the research and development of highly sensitive AFB_1 sensor [37], and biosensors with antibodies, enzymes, and nucleic acid aptamers as recognition elements were constructed. In addition, a large number of new materials have been used for the detection of AFB_1 at home and abroad.

As the research frontier of modern science and technology, nanotechnology has been widely used in the detection of AFB_1 abbreviation in foods in combination with electrophysiology, biology, and immunology and has become a development trend in the field of food safety research. The fabrication of Au nanostructures/graphene nanosheets modified ITO substrate has been reported; it is then used as a high sensitivity and AFB_1 sensor to detect very low concentrations of AFB_1 early by using Raman spectroscopy and electrochemical techniques [38]. Carbon dots (C-dots), as a new type of fluorescent nanomaterials, have attracted great attention in recent years due to their excellent light stability, good biocompatibility, low toxicity, and good water solubility. In order to expand the field of application, many researchers have studied the surface modification of C-dots with various functions [28]. Compared to traditional fluorescent sensors, C-dots-based aptasensors have greater potential because of their chemical inertness, ease of functionalization, and resistance to photobleaching. Based on the highly efficient fluorescence quenching properties of AuNP, a DNA sensor for detecting mRNA in living cells was developed. The results show that the fluorescence sensor based on AuNPs leads to high signal and sensitivity. CdTe quantum dots have the advantages of high fluorescence yield, strong photostability, long fluorescence lifetime, good biocompatibility, and wide excitation wavelength range. It is widely used in biomedical fields such as biochips, protein and DNA detection, and targeted tracing [39–42].

However, nanotechnology itself has its drawbacks. The preparation of CdS nanoparticles and CoOOH nanosheets takes a long time, and it is necessary to verify whether the synthesis is successful. In addition, the concentration of CdS nanoparticles directly affects PEC immunosensor; excessive CdS may lead to high background signal, whereas a low concentration produces a weak photocurrent response.

As an important medium, aptamer combines AFB_1 with nanomaterials and detects it by fluorescence and electrophysiological detection, which greatly shortens the time of detection and reduces the cost, and its accuracy and reliability. There is no change in sex, and the specificity and sensitivity are increased. However, since the AFB_1 aptamer preparation technique may still need improvement, as described above, the AFB_1 aptamer is an oligonucleotide, and the length of the small molecule nucleotide affects the binding to AFB_1. Aptamer Structure Switch coupled with horseradish peroxidase labeling on microplate for sensitive detection of small molecules. It is affected by the concentration of aptamer and AFB_1, and the results of Ye [24] show that when only 10 nM aptamer is used, the change in FP and AFB_1 concentrations has a good linear relationship between 0.05 and 5 nM of AFB_1. Coupled with the time required for preparation, combined with the complex and diverse detection methods, there is no standardized operation in life. However, based on the fluorescence and electrochemical detection of the AFB_1 aptamer, it is most promising for the detection of AFB_1.

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