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Review

Recent Advances in Ochratoxin A Electrochemical Biosensors: Recognition Elements, Sensitization Technologies, and Their Applications

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ABSTRACT: Ochratoxin A (OTA) is a class of mycotoxin that are mainly produced by Aspergillus and Penicillium and widely found in plant origin food. OTA-contaminated foods can cause serious harm to animals and humans, while high stability of OTA makes it difficult to remove in conventional food processing. Thus, sensitive and rapid detection of OTA undoubtedly plays an important role in OTA prevention and control. In this paper, the conventional and novel methods of OTA at home and abroad are summarized and compared. The latest research progress and related applications of novel OTA electrochemical biosensors are mainly described with a new perspective. We innovatively divided the recognition element into single and combined recognition elements. Specifically, signal amplification technologies applied to the OTA electrochemical aptasensor are proposed. Furthermore, summary of the current limitations and future challenges in OTA analysis is included, which provide reference for the further research and applications.

KEYWORDS: *ochratoxin A, electrochemical biosensor, aptasensor, sensitization technology, food safety*

INTRODUCTION

Mycotoxins are small organic molecule secondary metabolites produced by some fungi. They are highly toxic to humans and animals, which can enter the body through the skin, respiratory tract, and digestive tract. The ingestion of food contaminated with mycotoxins is the main way to enter the body. Ochratoxin (OT) is one of the mycotoxins produced by certain strains of Aspergillus and Penicillium. Currently, ochratoxin A (OTA), ochratoxin B (OTB), ochratoxin C (OTC), and ochratoxin α (OTα) are the main four members of the OT family.[1] Among them, OTA is the most widespread and toxic OT which was first discovered by Van Der Merwe et al. in 1965.[2] At present, OTA is widely found in a variety of products such as cereals, beans and their products, coffee, wine, grape juice, and dried fruit.[3] At the right temperature and moderation, OTA can be found in any species in agricultural areas around the world.[4] In addition, OTA is chemically stable and can survive at high temperatures and boiling. Thus, the OTA is particularly difficult to eradicate from the food chain. Because of the extensive existence and stable nature of OTA, the government has set strict restrictions on the content of OTA in food and feed. In general, OTA has an important public health significance. Therefore, real-time, sensitive, and in situ detection of OTA is important for control of OTA from contaminated foods into the human body. As shown in Figure 1, both developed and developing countries pay attention to OTA monitoring, and China plays a crucial role as a major grain producer (Figure 1A). Figure 1B demonstrates that OTA detection has received increased attention year by year, and it is always an important food hygiene issue.

Those efforts have contributed to the global development of various OTA analyses and detection platforms. Electrochemical biosensors (ECBs) convert the signals generated by the reflection of the target and the recognition element into electrical signals through the transducer to achieve fast, in situ, and cost-effective detection of targets, which have aroused widespread interest among researchers. The unique and rapid combination of recognition elements with OTA is the primary and vital step in the construction of OTA ECBs. Antibodies,[5] aptamers,[6] and biomimetic receptor-molecularly imprinted polymer (MIP)[7] play a major role. However, because of the trace presence of OTA, it is difficult to generate measurable signals merely based on the charge changes triggered by the combination of OTA and recognition elements. In order to avoid these problems, the application of modern sensitization technology in the signal transducer is particularly important. Because of the variability in shape, size, and composition, nanomaterial sensitization provides a new ascent for biosensors. Moreover, nucleic acid amplification technology and enzyme-assisted target recovery technology also provide a feasible strategy for OTA trace detection. To date, some comprehensive reviews based on the recent developments in OTA biosensors have been published.[8,9] For instance, Jiang et

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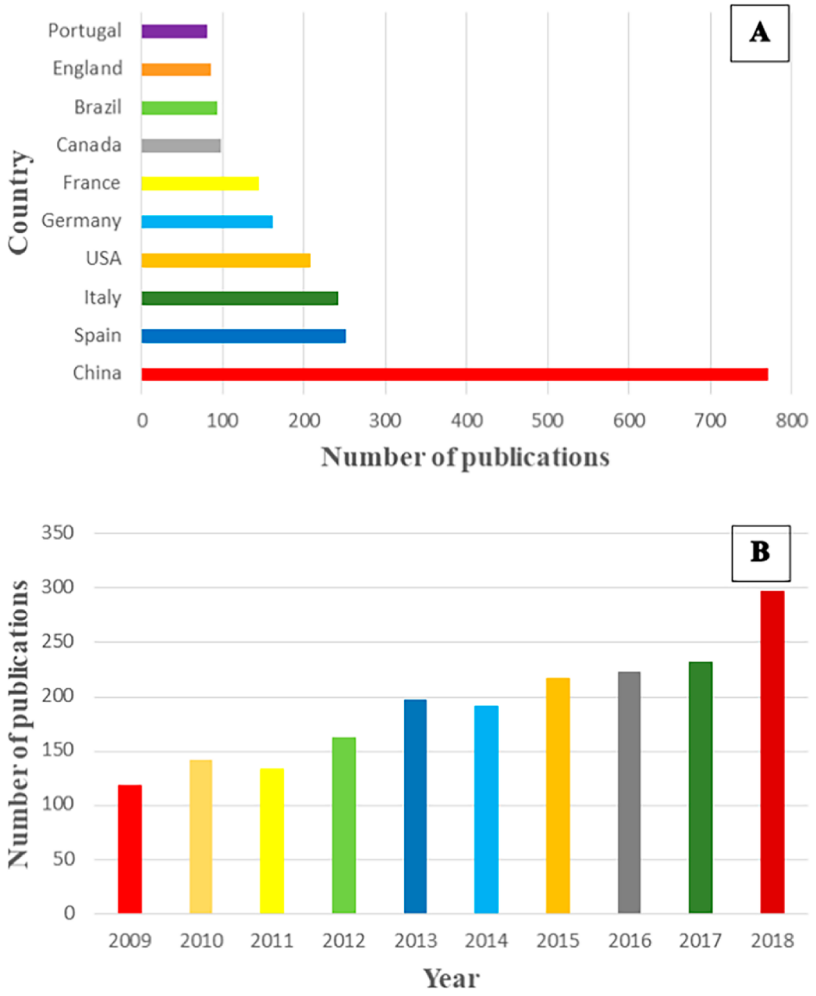

Figure 1. Publications on OTA detection by country (A) and year (B).

al.[8] summarized the progress in the application of nanomaterials in various OTA biosensors including ECBs. Subsequently, Alhamoud et al.[9] discussed the types of recognition elements and nanomaterials applied in OTA biosensors in more detail. However, the current summaries of the recognition elements in ECBs were not incomplete and did not involve the application of combined recognition elements (such as aptamer@DNAzymes). Admittedly, as the most potential recognition elements for the development of OTA ECBs, the aptamer has been the focus of researchers recently. Hayat et al.[10] discussed the progress of the OTA aptasensor with the nanomaterial signal amplification and switchable sensing platforms as the core topics. Shan et al.[11] reviewed the research advances of OTA electrochemical aptasensors according to different sensing strategies such as configuration transformation type, affinity type, and hybrid type. However, modern biotechnologies, such as nucleic acid amplification and nuclease-assisted techniques, have not been reflected in the current reviews, which is also an important means to improve the sensitivity of biosensors. Inspired by these works, herein, a new perspective is used to summarize the recent advances in the OTA electrochemical sensors. We innovatively divided the recognition element of OTA electrochemical biosensors into single recognition elements (antibody, MIP and aptamer) and combined recognition elements (Aptamer@DNAzymes, Aptamer@β-cyclodextrin, and Antibody@peptide). Meanwhile, on the basis of the current research highlights, we focused on the signal amplification technologies applied in OTA electrochemical aptasensors. For the first time, we systematically classified signal sensitization methods into material sensitization, technical sensitization, and combinative sensitization. Furthermore, an overview of the latest applications of OTA electrochemical sensors is given. Finally, the current limitations and future challenges in OTA detection and analysis are discussed as well.

IMPORTANT KNOWLEDGE OF OCHRATOXIN A

Metabolic Pathways. Metabolic studies are helpful to elucidate the pharmacological effects and toxicity of OTA, and to better understand the metabolism of OTA in animals and humans. At the same time, they provide a new direction for the detection of biomarkers of OTA contamination. In recent years, a large amount of different experiments on the metabolism of OTA in vivo and vitro have been conducted. Figure 2 systematically summarizes the major metabolic process (phases 1 and 2) and resulting metabolites in different species. The phase 1, the primary metabolic pathways of OTA, consists of hydroxylation, hydrolysis, lactone opening, conjugation, and dechlorination according to many studies.[12,13] And the main metabolites of OTA are OTα (violet line), 4(R)-OH-OTA, 4(S)-OH-OTA, 10-OH-OTA (blue line), OTB (green line), and OP-OTA (red line),[14,15] of

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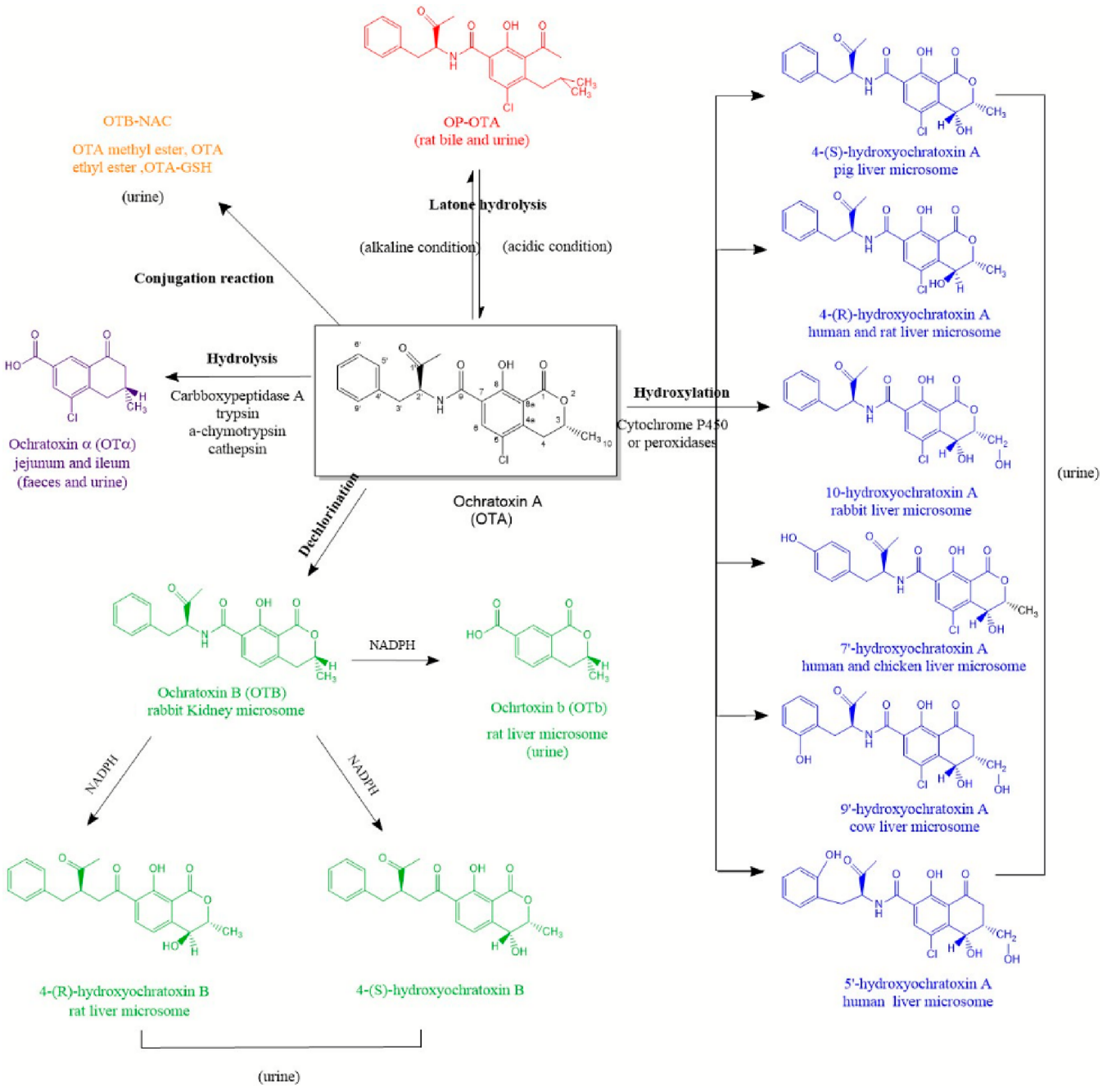

Figure 2. Main metabolic pathways of OTA (the dotted arrow represents the metabolic process of phase 2).

which the lactone-opened product OP-OTA is more highly toxic than its prototype.[12] OTA cleaves peptide bonds to form OTα by a hydrolysis reaction.[16] Liver microsomes of humans, pigs, and rats are capable of metabolizing OTA by cytochrome P450 to two epimers, 4(R)-OH-OTA and 4(S)-OH-OTA. 4(R)-OH-OTA and 4(S)-OH-OTA are mainly formed in humans and rats and pig microsomes, respectively.[17] It has been reported that OTA can be cultured in rabbit liver microsomes to produce another hydrolysate, 10-OH-OTA, which is also found in human bronchial cells and rabbit kidney microsomes in vitro, but not in vivo.[18] Under alkaline conditions, OTA can be hydrolyzed into lactone-opened OP-OTA, which was first detected by Xiao et al. and characterized by higher toxicity.[19] OTB is formed by dechloridation of OTA, which can be hydrolyzed into 4-OH-OTB or OTb in liver microsome by MADPH.[20] In addition, 9′-OH-OTA, 7′-OH-OTA, and 5′-OH-OTA were first detected by Yang et al. in 2005.[21] At present, the reaction mechanisms of phase 2 are not fully understood, but researchers have made some progress understanding phase 2 metabolites of OTA in the vivo and vitro. In 2016, OTA glucuronide conjugate metabolite has been determined in infants and adults' urine samples by using indirect measurements.[22] In the next year, a vitro coculture system was established to analysis of OTA metabolites produced by Caco-2 and HepG2 cells. The result indicated that OTB, OTA methyl ester, OTA ethyl ester, and the OTA glutathione conjugate (OTA-GSH) were major metabolites of the system.[23] The last three are conjugated metabolites. Moreover, OTA methyl ester was considered as a novel exposure biomarker of OTA. Recently, for the first time,

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researchers used direct analytical methods to detect ochratoxin-*N*-acetyl-L-cysteine (OTB-NAC).[24]

Multiple Toxicities. As early as 1993, International Agency for Research on Cancer (IARC) classified OTA as a Class 2B carcinogen (human probable cancinogen) with toxicity second only to aflatoxin. Current studies have also confirmed that OTA has multiple toxicities, such as nephrotoxicity, hepatotoxicity, immunotoxicity, neurotoxicity, and cytotoxicity.[25] The kidney is one of the primary metabolic organs of OTA; hence, renal damage is a common adverse consequence of long-term OTA intake. OTA was first discovered in the Balkans and was considered to be one of the main pathogenic factors of Balkan endemic nephropathy (BEN).[26] Previous studies have shown that, to some extent, reabsorption in the kidney proximal and distal tubules kidney contributes to an increase in the concentration of OTA in the kidney tissue, which increases the susceptibility of the kidney.[27] It is worth mentioning that OTA is nephrotoxic to all monogastric mammal species but not to adult ruminants.[28] The liver is another primary target organ of OTA biotransformation.[29] Through experimental research, Felizardo et al. found that long-term exposure to high levels of OTA might be associated with a high risk of hepatocellular carcinoma (HCC) occurrence.[30] It also has been reported that OTA-mediated oxidative damage can impact lipids, proteins, and DNA, alter antioxidant status, and inhibit the synthesis of proteins and DNA, which were thought to induce genotoxicity, cytotoxicity, and immunotoxicity in vivo and in vitro.[13]

In addition, studies have shown that OTA toxicity may be associated with its metabolic processes and oxidative stress, but the exact mechanism was unclear.[13] The toxicities of most of metabolites, such as OTα, 4-OH-OTA, 10-OH-OTA etc., are lower than OTA, which is beneficial for elimination of OTA in vivo. However, there is also a small proportion of metabolites that are more toxic than OTA, such as OP-OTA. Therefore, in-depth study of the metabolic process is of great significance to protect human health after ingestion of OTA.

Limit Standards. Because of the adverse effects of OTA on animal and human health, many countries have developed regulations on the maximum concentration of OTA concentration in contaminated food and feed in recent decades to ensure food and feed security. Table 1 summarizes the standard of OTA contamination limit enacted by China,[31] European Union (EU),[32] and Codex Alimentarius Commission (CAC).[33] The focus of different standards is slightly different, mainly reflected in the following two points: (1) the limit standard of processed grain and raw grain in China is uniform, while in EU, the limit of raw grain is lower than processed grain. (2) There are no provisions for cereals, infant foods, and dietary foods for special medical purposes in China, while strict restrictions are imposed by the EU. In general, the standards set by the EU are more mature and complete.

Table 1. Limit Standards for OTA in Food and Feed in China,[31] EU,[32] and CAC[33]

region or organization	food or feed category	limit standard (μg/kg)
China	cereal and their products	5.0
	beans and their products	2.0
	wine	5.0
	roasted coffee beans	5.0
	grinding coffee	5.0
	soluble coffee	10.0
	compound feed	100.0
	cereal and their products (feed)	100.0
European Union	raw grain feed	5.0
	processed cereal products and direct human consumption of cereals	3.0
	dried fruit	10.0
	roasted coffee beans and grinding coffee	5.0
	soluble coffee	10.0
	wine, fruit wine, and grape juice	2.0
	processed cereals and infant foods	0.50
	dietary foods for special medical purposes for infants	0.50
CAC	wheat, barley, and rye	5.0

CONVENTIONAL DETECTION METHOD

OTA usually exists in food in trace amounts, so it is necessary to quantitatively determine the concentration of OTA in food and feed by effective and accurate detection methods. Conventional detection methods of OTA include immunoassay, chromatographic analysis, optical biosensors, and biochips. The immunoassay utilizes specific binding responses of antigens and antibodies to analytical targets, which require various labels. According to the type of labels, it can be divided into enzyme-linked immunosorbent assay (ELISA),[34−36] colloidal gold immunoassay (CGIA),[37] fluoroimmunoassay (FIA),[38,39] chemiluminescence immunoassay (CLIA),[40] etc. They can complete the analysis in a relatively short time and mainly be used for rapid detection of OTA and screening of large samples. Among them, ELISA is one of the most common methods, which has low cost and wide applicable range, but it is cumbersome and time-consuming, and there are many factors affecting the reaction. For chromatographic analysis, based on the principle of the dissolution in the similar material structure, different substances successively leave the column, and different peak signals are obtained in the detector. Thus, it is able to accurately analyze the concentration of the various target after it is extracted from matrix, which mainly consists of thin layer chromatography (TLC),[41,42] gas chromatography (GC),[43,44] high-performance liquid chromatography (HPLC),[45] and so on. They usually have good repeatability and high accuracy. Among them, HPLC has absolute superiority in the international standard system and has been widely used in laboratories at home and abroad. In general, HPLC is equipped with a fluorescence detector (FLD)[46,47] or used in conjunction with mass spectrometry (MS) to detect OTA. However, chromatographic methods have higher requirements for equipment, operators, and sample pretreatment, and the process is complicated and is only suitable for the appropriate laboratory analysis. In addition, they usually require the use of organic solvents, which imposes an additional burden of environmental pollution. Because of their simple operation, high sensitivity, and fast response, optical biosensors have been used in the field of OTA detection in food recently. It is quantified by the change of optical signal (intensity, color, refractive index, etc.) caused by the reaction between the target and the detection reagent. According to the detection technology, optical biosensors can be divided into fluorescence,[48] colorimetric,[49] chemiluminescence (CL),[50] surface enhanced Raman scattering (SER),[51] and local surface plasmon resonance (LSPR)[52] sensors. Nanomaterials and enzyme-assisted signal amplification techniques had been applied in optical sensors to build

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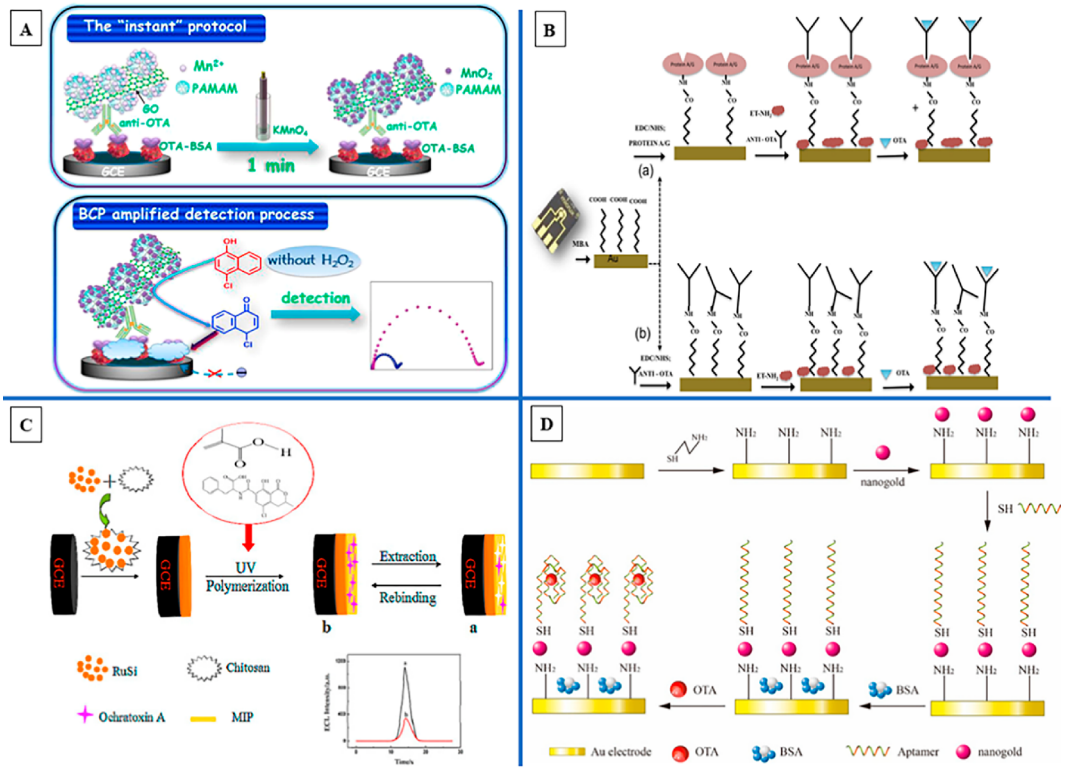

Figure 3. Schematic diagrams of OTA ECBs based on single recognition element: (A) antibody (labeled),[63] (B) antibody (label-free),[66] (C) MIP,[69] and (D) aptamer.[75]

sensitive optical sensing platforms for rapid OTA detection.[53,54] However, poor stability and insufficient miniaturization of equipment are the two main shortcomings of existing optical biosensors. With the advantage of high throughput, rapid analysis, and miniaturization, biochip is an analysis technique fixing a large number of biometrics molecules to the surface of a carrier (such as silicon wafer, glass slide, etc.) to quantify the target molecules by using the specific affinity reaction of biomolecules. It has great application prospects in the simultaneous detection of mycotoxin. However, it is easy to cause sample contamination during the amplification reaction of the sample target, which reduces the sensitivity of this method. In addition to the detection of OTA itself, the conventional detection method can also be applied for the analysis and detection of a variety of OTA metabolites. However, there are relatively few reports on the detection of OTA metabolites and was mainly based on the use of LC-MS/MS and HPLC/FLD.[55]

On the basis of the above-mentioned summary, the traditional detection method has been unable to meet the requirements of modern, fast, on-site, and simple detection and analysis. Therefore, the novel detection method with the characteristics of high sensitivity, good selectivity, fast, green, and simple operation need to be further developed.

NOVEL ELECTROCHEMICAL BIOSENSING METHOD

In recent years, the application of ECBs in OTA detection has developed rapidly. The detection principles of ECBs are based on the current change generated by the redox reaction of electrode surface caused by the combination of biorecognition elements and targets. Therefore, the ECBs have the advantages of both biochemical reactions and electrochemical transduction, and have high specificity, affinity, and inherent sensitivity and reactivity. Furthermore, excellent portability and stability, affordability, fewer reagent consumption, no need for complex preprocessing, and superiority of miniaturization are also the outstanding advantages of the ECBs. In combination with the characteristics of OTA and the requirement of modern testing, at present, ECBs are the potential method of OTA concentration analysis.

Undoubtedly, as a member of the biosensors, the recognition of targets and the construction of a signal transduction platform are two primary tasks of the ECBs. That is, these refer to the selection of recognition elements, electrode modification, and detection strategy of the electrical signal. In general, the ECBs can be divided into different categories according to the selected recognition elements.[56,57] Recently, various ECBs have been developed by researchers at home and abroad using a single (antibody, aptamer, etc.) or combined (aptamer@DNAzyme etc.) recognition element.

ECBs Based on Single Recognition Element. Antibody, MIP, and aptamer are the three main recognition elements of the OTA ECBs. A schematic diagram of ECBs based on a single recognition element is shown in Figure 3.

Antibody. Electrochemical immunosensors are mainly based on the specific recognition ability of antigen−antibody to achieve qualitative and quantitative analysis. OTA, as a small and nonimmunogenic molecule, can specifically bind to appropriate antibody. At present, anti-OTA antibody mainly includes polyclonal antibody, monoclonal antibody, and recombinant antibody.[58,59] According to whether the label materials are used in the process of immunoassay, they can be classified as nonlabeled immunosensors and labeled immunosensors.

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Labeled immunosensors: According to the type of antigen−antibody immune response, the analytical principles of electrochemical immunosensors can be divided into two types: competitive methods and sandwich methods. The former may be further discriminated into direct and indirect competition methods. The direct competition method mainly involves the process of the enzyme-labeled antigen competing with the target antigen to bind to the antibody immobilized on the electrode surface, which can lead to a change in the current signal. Alkaline phosphatase (ALP)[21,60] and horseradish peroxidase (HRP)[61,62] are commonly used labels. However, the enzyme is easily inactivated, and its catalytic efficiency is low. These inherent defects restrict the development of enzymes as labels. At the same time, in recent years, nanomaterials as labels have attracted the interest of researchers. On the basis of the direct competition method, Tang et al.[63] constructed an OTA impedance immunosensor by using an instantaneous catalyst to amplify the electrical signal. As shown in Figure 3A, graphene oxide nanosheets (GO)-amine-terminated dendrimer (PAMAM) labeled anti-OTA could not only cause a competitive reaction between target OTA and the OTA-BSA fixed on the electrode but also could combine a mass of Mn^{2+}, which can in situ form MnO_2 with an extremely strong catalytic performance. Thus, the sensitivity of the analytical method was improved, which leads to a low LOD of 0.055 pg/mL and a wide linear range between 0.1 pg/mL and 30 ng/mL. In terms of indirect competition methods, the antigen immobilized on the electrode surface and the antigen to be detected compete with the labeled antibody in the solution. Heurich et al.[64] designed a disposable indirect competitive electrochemical immunosensor method, with horse radish peroxidase (HRP) as the label to catalyze TMB/H_2O_2 to generate the current signal. When OTA existed, fewer HRP-labeled antibodies were immobilized on the surface of electrode. By recording the change of the chronoamperometry, a LOD of 0.05 μg/L and a linear detection range from 0.01 to 100 μg/L were achieved. A sandwich method is an immunoassay method based on immobilized antibody, antigen, and labeled antibody to form a sandwich immune complex. On the basis of this principle, an ECB was designed for quantitative detection of OTA and was successfully used in red wine sample. Octahedral gold nanoparticles (Oct AuNPs) with edge and sharp corners are used subtly for sensor design. First, Oct Au NPs as substrate materials were modified on GCE to provide more position to immobilize primary amine groups of antibodies. Moreover, Au octahedron plasmonic colloidosome was another form of Oct Au NPs produced in inverse emulsion. It could immobilize toluidine blue (TB) and secondary antibodies to obtain a mixture that acted as a label to amplify the signal and improve the sensitivity and stability of the sensor. Thus, positive correlation between SWV signal and OTA concentration in matrix was obtained with the linear range from 0.1 pg/mL to 10 ng/mL. When the immunosensor was applied in spiked wine samples, an average recovery of 99.6−100.1% was achieved.[65]

Nonlabeled immunosensors: Compared to other immunosensors, nonlabeled immunosensors mainly detect analytes by the changes in electron transfer impedance caused by antigen−antibody reactions. Since there is no need to label antigens or antibodies, the preparation process of this kind of immunosensors is simple, but the signal amplification means are limited. It is worth noting that immobilization of biomolecules is a key step in the construction of the immunosensors. As shown in Figure 3B, Malvano et al.[66] compared two antibody immobilization methods (oriented and not oriented) for OTA detection. According to the research results, the selectivity and sensitivity of biosensors based on oriented immobilization methods (protein A/G was used to fix on the antibody) are better than nonoriented immobilization methods (protein A/G not used). The protein A/G was able to immobilize antibody to keep antigen-binding sites outward. Therefore, oriented immobilization was a preferred method with a linear range of 0.01−5 ng/mL. This method also was successfully applied to spiked cocoa bean samples, and the results were consistent with an ELISA kit.

In summary, the electrochemical immunosensors exhibit good performances in OTA analysis, such as high specificity, good reproducibility, and low LOD. However, a primary imperfection of the biosensors is cross-reactivity of antibodies. Thus, it is of great importance to explore and develop other types of molecular recognition elements.

MIP. MIPs are considered to be economical, attractive, and powerful polymer materials that mimic antibody function.[67,68] MIPs are polymer receptors synthesized by different monomers, which is widely used in sensors, catalytic reactions, and separation because of their high affinity in recognizing and binding specific target molecules. As a bionic recognition element compared with biological-based recognition receptors (such as antibodies and aptamers), MIPs' most prominent advantages are physical and chemical stability and manufacturability.

Many MIP-based ECBs have been designed for OTA detection, especially in 2015 and 2016. Most of the MIP-based ECBs combined nanometer materials due to the good properties of nanomaterials as mentioned above. As shown in Figure 3C, an electrochemiluminescence (ECL) sensor using MIPs as a recognition element was designed to detect OTA in corn. In the work, $Ru(bpy)_3^{2+}$-doped silica nanoparticles (RuSi NPs) were employed to modify GCE, which can emit an ECL signal. When the MIP hole was filled by the target OTA, electron transfer on the surface of the GCE was hindered, and the ECL signal was lowered. The linear range was from 0.1 pg/mL to 14.76 ng/mL, and the LOD was 0.027 pg/mL. For the detection of spiked corn samples, this MIP-ECL biosensor showed a recovery from 95.3% to 99.3%, and an RSD from 5.2% to 6.1%, which exhibited good accuracy and practicability.[69] On the basis of similar principles, quantitative detection of OTA concentration was achieved by measuring the change of the DPV signal before and after OTA filling of holes. Also, this MIP sensor had been successfully used in OTA on-site analysis in beer and wine samples with a recovery close to 100.0%.[70]

However, at present, the selectivity of MIPs is insufficient to compete with biogenic recognition receptors, and the sensing strategy based on these ECBs is inadequate. In addition, large-scale preparation of MIPs using OTA as a template is expensive.

Aptamer. Aptamer (also be called "chemical antibody") is a type of oligonucleotide fragment (synthetic single-stranded DNA or RNA) that binds to a target molecule (ion, protein, toxin, nucleic acid, whole cell, etc.) with high specificity in vitro by phylogenetic analysis of exponential enrichment (SELEX).[71] Since an amplification step is involved in the screening process, this gives the aptamer some advantages over other recognition elements, such as wide recognition range,

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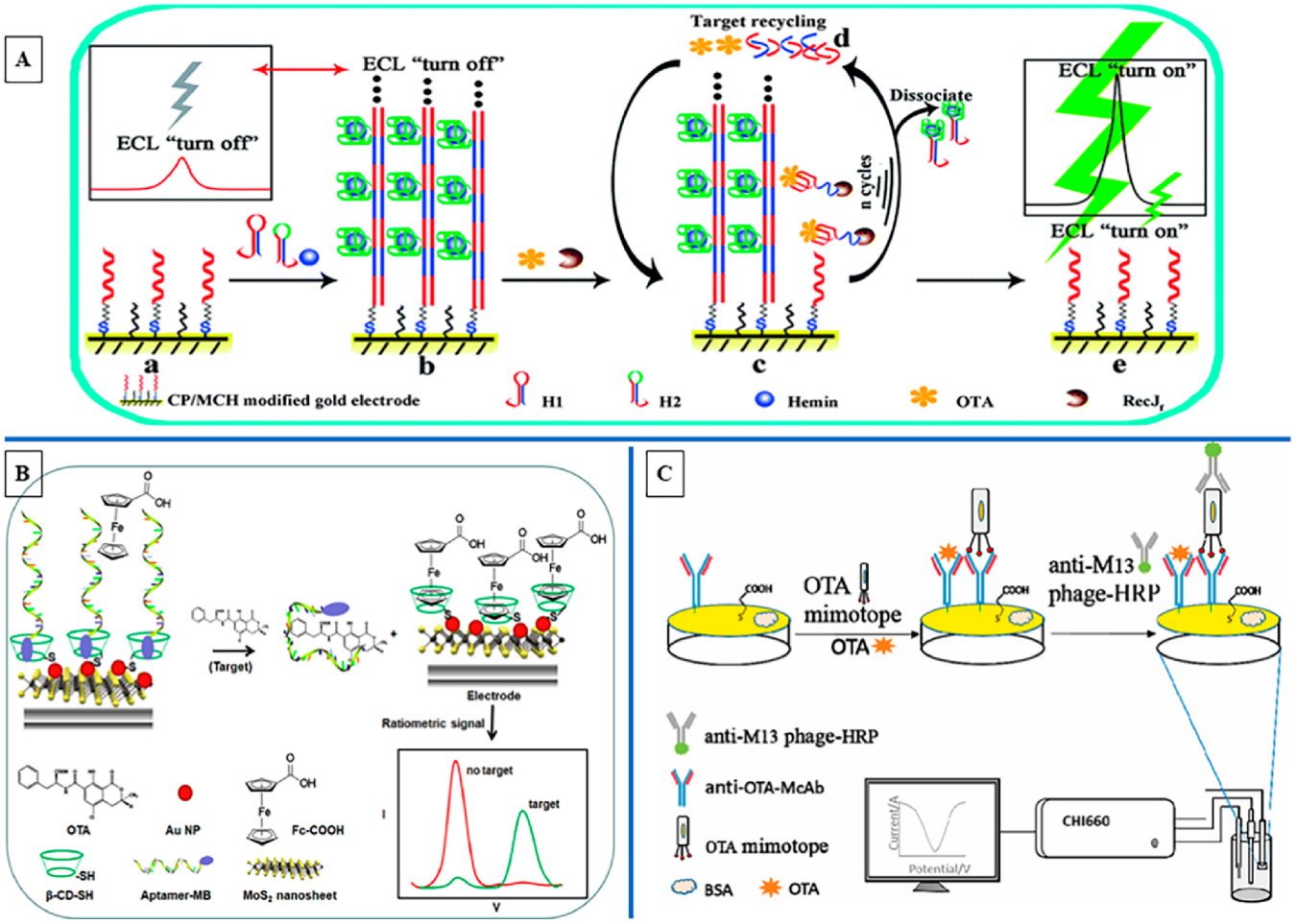

Figure 4. Schematic diagrams of OTA ECBs based on a combined recognition element: (A) aptamer@DNAzyme,[77] (B) aptamer@β-CD,[79] (C) antibody@peptide.[83]

easy preparation and synthesis process, easy to modify, high affinity, stability, and specificity.[72] After several rounds of screening, the scientists screened out seven sequences of aptamers with high affinity.[11] Considering the number of bases, the K_d value, and compatibility with the sensing platform, the most widely used aptamer sequence in OTA electrochemical aptasensor currently is aptamer 1.12.2 (GATCGGGTGTGGGTGGCGTAAAGGGAGCATCGGACA).[73] Besides, compared with complex processing methods such as antibody immobilization, solid−liquid separation and washing, aptamers can be applied to homogeneous detection with various signal amplification techniques to achieve highly sensitive detection.[74]

As shown in Figure 3D, a label-free electrochemical aptasensor was developed for OTA analysis. By layer-by-layer self-assembly, the aptamer was immobilized on the Au by Au−S bond. After the introduction of OTA, the combination of aptamer and OTA would increase the resistance of $[Fe(CN)_6]^{3-/4-}$ diffusion, resulting in the change of electrochemical impedance spectroscopy (EIS) being proportionable to the concentration of OTA. The linear range was from 0.1 to 10.0 ng/mL with an LOD of 0.030 ng/mL.[75]

Studies have shown that aptamers have high affinity, good selectivity, low expenditure, long shelf life, wide range of detection, and the ability for simultaneous detection of multiple substances.[76] This may be the reason why researchers are paying more and more attention to aptasensors.

ECBs Based on Combined Recognition Elements. On the basis of a single recognition element, combined recognition elements such as aptamer@DNAzymes, aptamer@β-cyclodextrin, and antibody@peptide also exhibit excellent recognition performance. The schematic diagrams of ECBs based on combined recognition elements are summarized in Figure 4.

Aptamer@DNAzymes. DNAzyme refers to a complex having peroxidase activity consisting of G-quadruplex and hemin. Compared with HPR, DNAzyme has the advantages of high chemical stability, low cost, and simple synthesis method. It is attracting great attention as a catalytic and has a wide range of applications in analysis. Aptamer@DNAzymes as a combined biorecognition element has been widely used in various OTA biosensors. In this type of ECB, aptamer can combine with OTA with high affinity and specificity as target recognition element, while DNAzyme have excellent catalytic performance as signal amplification element. As shown in Figure 4A, an ECL "turn-on" biosensor was developed for OTA detection by aptamer@DNAzyme supersandwich nanostructures. In this approach, the formation of supersandwich nanostructures could quench the ECL emission. In the presence of OTA, the aptamer@DNAzyme supersandwich nanostructures were separated, and the ECL emission was restored accordingly, resulting in a linear range from 0.2 pg/mL to 1 ng/mL. Also, target recovery of exonuclease (Exo) increased the sensitivity of detection with LOD of 75 fg/mL. In order to detect red wine samples, three different

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Table 2. Material Sensitization of OTA Electrochemical Aptasensors Based on Single Nanomaterials[a]

type of nanomaterials	electrode modification	function of nanomaterials	signal tag	detection method	sensing strategy	LOD	linear range	sample	reference
AuNPs	CA/AuNPs/Au	electrode modification		EIS	direct detection	0.030 ng/mL	0.1−10 ng/mL	red wine, white wine, grape juice	75
	cNDA1-AuNP-cDNA2/AuNPs-cDNA3/Au	carry signal probe, catalyst	Fc	DPV	competitive binding	0.001 ppb	0.001−500 ppb	red wine	85
	DNA1/DNA2/G-rich DNA3-GNPs/DNA4/Au	carry signal probe	MB	DPV	sandwich detection	0.75 pM	2.5 pM to 2.5 nM	wine	114
	DNA1/DNA2/DNA3-f-AuNPs/GC	electrode modification	MB	CV	competitive binding	30 pg/mL	0.1−20 ng/mL	red grape wine	123
AgNPs	APT/cDNA/AgNPs/PDANS/Au	carry signal tag	SA	DPV	competitive binding	0.57 pM	10^{-6} to 10^{-2} μM	red wine	95
MoS_{2*}	APT/cDNA/Au	catalyst	MoS_2	amperometric	replacement method	0.23 pg/mL	0.5pg/mL to 1.0 ng/mL	red wine	124
	APT/GO/SPCE	catalysis	GO	DPV	direct detection		310 fM to 310 pM		125
	cDNA1/APT/cDNA2/SPCE	carry signal tag	SWCNTs	CV	competitive binding	52 pM	180 pM to 30 nM	serum, grape juice	126
CBNs	CS-f-GR/SA/ATP/ITO	electrode modification		DPV	direct detection	1 fg/mL		grape juice	89
	APT/g-C_3N_4/ Au	catalysis		CV	competitive binding	0.073 nM	0.2−500 nM	red wines, juices, corn	91
MNNs	MBs/GC	electrode modification	ALP	DPV	competitive binding	0.11 μg L	0.11−15 μg/L	wine	127
QDs	Cy5-pDNA/cDNA/CdTe/GC	ECL emission		ECL	Direct detection	0.17 pg/mL	0.0005−50 ng/mL	corn	95

[a]Cysteamine (CA), graphene (GR), streptavidin (SA), chitosan (CS), screen printed carbon electrode (SPCE).

concentrations of the target OTA were added, and this method showed high consistency with the ELISA kit.[77]

Aptamer@β-cyclodextrin. β-Cyclodextrin (β-CD) has a special molecular structure of "internal hydrophobicity, external hydrophilicity" and has a strong enveloping effect with many molecules. In recent years, it has been widely applied in electrochemical sensors because it can induce MB, Fc, and other electroactive substances close to the electrode surface via host−guest recognition to generate a strong electrical signal. Wang et al.[78] designed a novel ECB based on host−guest recognition of β-CD and Exoassistant. β-CD was immobilized on the surface of the GCE, which could enrich the MB. During hybridization of DNA, detection of the target and hydrolysis of Exo occurred in solution. This homogeneous electrochemical biomolecule recognition technology based on β-CD host−guest recognition could avoid the cumbersome operation of the immobilization process of aptamers. By recording the change of peak current, a linear range from 10 pg/mL to 10.0 ng/mL and a LOD of 3 pg/mL were achieved. Aside from homogeneous detection, ECBs based on the β-CD could achieve a dual-signal for OTA detection. As shown in Figure 4B, in the absence of OTA, MB-aptamer gathered on the surface of the β-CD/MoS_2 modified electrode and exhibited a strong redox current of MB. When OTA was introduced, the MB-aptamer combined with OTA resulted in a decrease of the redox current of MB and an increase of the redox current of Fc. This "dual-signal" strategy effectively improved the sensitivity of the biosensor, Thus, a linear range from 0.1 to 50 nM and LOD of 0.06 nM were obtained.[79]

Antibody@peptide. At present, peptides as a "green" auxiliary recognition element are introduced in OTA electrochemical immunosensors. In the traditional competitive detection of immunosensors, the target OTA needs to be prepared competing with the labeled-OTA to bind antibodies, which increases the health risks of humans.[80] Inspired by this, screening for an alternative antigen can reduce the risk of toxicity to researchers and the environment. Phage display peptide technology can be used to screen for mimic epitopes of small molecule haptens of OTA to prepare antigen substitutes by panning. Moreover, mimotopes can increase the sensitivity of the biosensors.[81] And phage display peptide technology has been successfully used by researchers for the analysis of other mycotoxins, such as deoxynivalenol (DON).[82]

Recently, Hou et al.[83] developed a green electrochemical immunosensor for OTA ultrasensitive detection with the assistance of phage displayed mimotope peptide. As demonstrated in Figure 4C, OTA mimotope competes with target OTA for binding anti-OTA. Then the OTA mimotope could combine with HRP conjugated anti-OTA to form a sandwich immune complex to generate signal detection by the SWV technique. Moreover, the SWV response depended linearly on the OTA concentration and was inversely proportional to the concentration of OTA from 7.17 to 548.76 fg/mL. The LOD was 2.04 fg/mL. The recoveries of spiked OTA in corn and beer samples based this assay were 89.6−107.4% and 99.3−104.3% respectively.

In summary, various types of single and combined recognition elements have been designed for OTA analysis in food samples. Combined recognition elements partly remedy the imperfection of the low sensitivity of ECBs based on single recognition elements, and new green recognition elements such as peptide are receiving increasing interest from researchers. However, these technologies are immature and cumbersome. At present, because of the high affinity, inexpensive in vitro synthesis, easy to modify, switchable conformation, and molecular mass much smaller than other biometric molecules such as antibodies or MIP, there has been a great potential in the development of ECBs based on aptamers.

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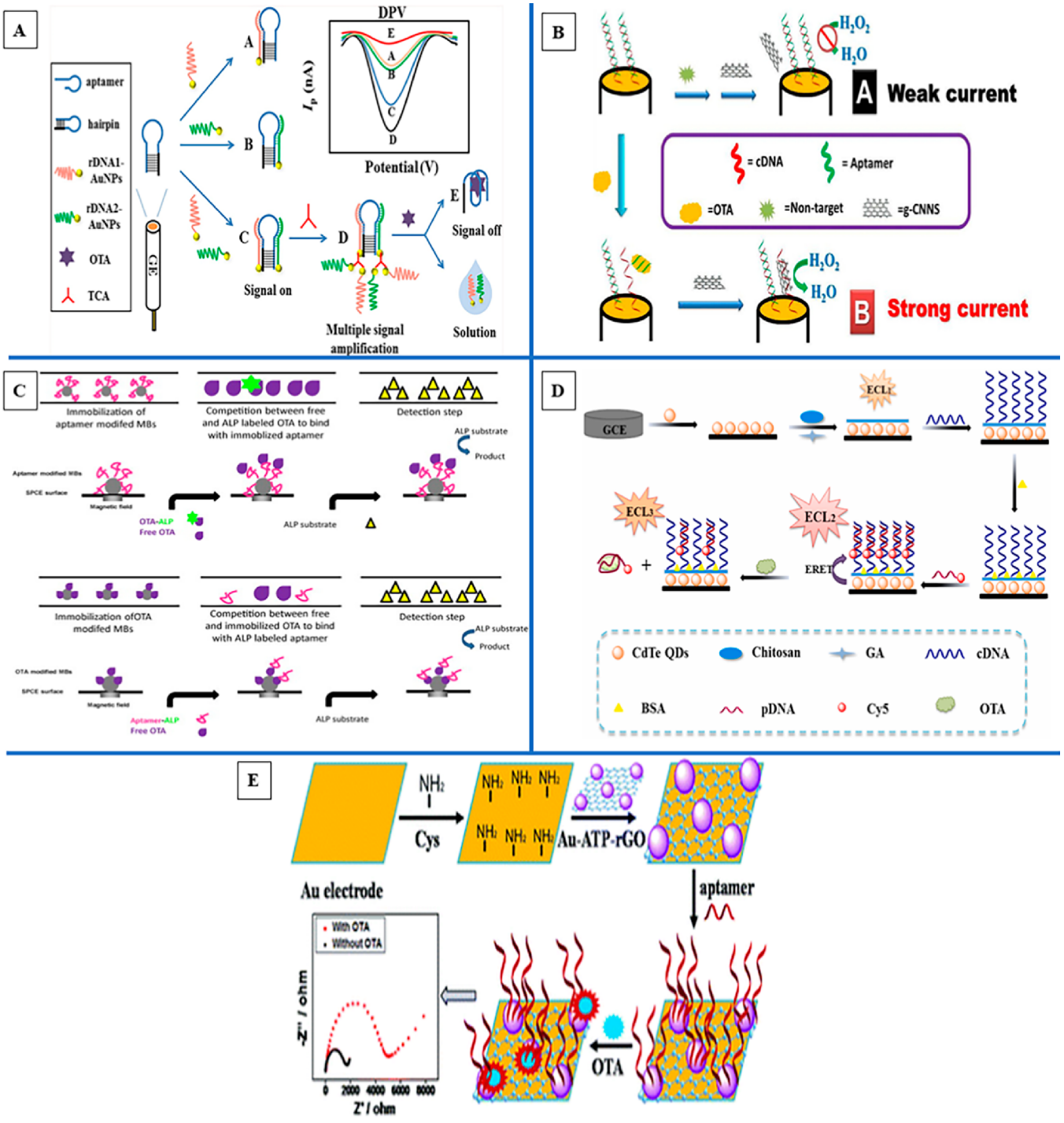

Figure 5. Schematic diagrams of material sensitization of OTA electrochemical aptasensor: single nanomaterial (A–C)[84,91,93,95] and multiple nanomaterials (D–E).[102]

ELECTROCHEMICAL APTASENSOR SENSITIZATION TECHNOLOGY

Electrochemical aptasensors have been extensively employed for the analysis of OTA. Generating an obvious change of electrochemical signals between the electrode interface and the transducer for electrochemical detection of OTA is necessary. Nevertheless, it is insufficient to generate measurable change only by combining the OTA with the aptamer. To meet the modern requirements of OTA trace detection in contaminated food and feed samples, sensitization technologies are needed in the progress of constructing electrochemical aptasensors. To date, sensitization technologies of electrochemical aptasensors can be classed into material sensitization (material-based signal amplification), technical sensitization (technology-based signal amplification), and combinative sensitization.

Material Sensitization. With the rapid developments of nanotechnology, the emergence and applications of nanomaterials with special magnetic, optical, and conductive properties are profoundly influencing the field of analysis. They have the advantages of ease of synthesis, flexible size controllability, large specific surface area, good electrical conductivity, and

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easy binding to biomolecules. These advantages provide new ideas for the research of OTA electrochemical aptasensors. Various nanomaterials have different signal amplification pathways such as typically playing the role of electrode modification (promoting electron transfer and immobilization of biomolecules), labeling biomolecule, and catalyst.

Nanomaterials with good electrical conductivity, such as metal nanoparticles (MNs) and carbon nanotubes, can be used as a bridge to connect biomolecules and electrodes, and can accelerate the electron transfer rate of interphase interfaces. Because of the surface effect of the nanoparticles and the small size effect, the nanoparticle-modified electrode has a larger specific surface area and thus has more active sites, which increases the amounts of aptamer immobilized on the electrode surface. In view of the above two points, nanomaterials are excellent modification materials of electrodes. In addition, the use of nanomaterials as markers for biomolecules can greatly improve the performance and significantly increase the sensitivity of aptasensor. Electrocatalysis is one of the most important applications of nanomaterials in the field of ECBs, and excellent electrocatalytic activity for certain reactive substances can effectively amplify the electrochemical signal. The use of single nanomaterials to amplify detection signals in OTA electrochemical aptasensors is shown in Table 2.

Metal Nanomaterials. Metal nanomaterials (MNs) refer to metals, metal oxides, bimetallic nanoparticles, and transition metal sulfides (TMSs) with nanostructures, which are novel multifunctional materials that organically combine the physicochemical properties of metals with the properties of nanomaterials. MNs not only have the general properties of nanomaterials, but their unique catalytic activity gives them have great application prospects in the construction of ECBs. In recent years, with respect to the application of metal nanomaterials in electrochemical aptasensors, noble metal nanomaterials are particularly prominent. Normally, AuNPs and AgNPs are two most widely used nanomaterials. AuNPs as an electroactive label or a carrier material for biomolecules are an efficient and feasible signal amplification strategy for OTA electrochemical aptasensors. AgNPs is cheaper except for the above properties, but less stable and modifiable than AuNPs.

Our research group established an ultrasensitive signal−on−off electrochemical method for OTA analysis based on the hairpin aptasensor via a multiple signal amplification strategy (Figure 5A). The hairpin aptamer was able to combine double AuNPs labeled signal probes, contributing to a strong DPV signal (signal-on). In the presence of OTA, the specific binding of the aptamer to the OTA led to the separation of the signal probe from the electrode, resulting in the decrease of the DPV signal (signal-off). This method achieved a high level of sensitivity with a low detection limit of 0.5 pg/mL and a wide linear range of 1 pg/mL to 1 ng/mL. The sensor was successfully applied to the detection of roasted coffee bean samples, and the recovery of OTA was 97−100.6%.[84] Chen et al.[85] constructed an ultrasensitive dual AuNPs conjugate electrochemical aptasensor for OTA determination. The major function of first AuNPs was loading more auxiliary probe, and the second AuNPs was loading more signal probe. This sensing strategy results in a low detection limit of 0.001 bbp and a wide linear range over 6 orders of magnitude. Zhang et al.[86] proposed an ultrasensitive OTA electrochemical aptasensor. In this work, AgNPs were deposited on polydopamine nanospheres (PDANSs) in situ, forming AgNPs/PDANSs as signal probe to carry streptavidin (SA). SA as a signal label could bind to the terminal biotin of the aptamer. After the introduction of OTA, the signal probe was replaced, resulting in a lower current signal. The current change depended linearly on the concentration of OTA in the range from 1pM to 0.02 μM, with an LOD of 0.57 pM.

Carbon-Based Nanomaterials. Because of the excellent optical, electrical, magnetic, and mechanical properties of carbon-based nanomaterials (CBNs), it plays an important role in energy, environment, photovoltaic composites, biomedicine, and other fields. As the structures of the CBNs reach nanometer size, their properties will change peculiarly with the size and surface chemistry. The common CBNs are mainly in several forms: fullerenes, carbon nanotubes, carbon nanofibers, nanodiamonds, GR, and carbon dots. Among them, carbon nanotubes[87,88] and GR[89,90] are frequently used in the field of electrochemical detection. Carbon nanotubes have a large specific surface area, the surface is rich in π-electrons, and the pore size is controllable during preparation, so it is an ideal electrode material. As a new member of the CBNs family, GR has excellent conductivity, high mechanical strength, and a large specific surface area. It is a new electrode modification material superior to carbon nanotubes, attracting electrochemical analysis researchers' great research interest. Applied to the detection of OTA, CBNs are generally used in combination with other nanomaterials, especially AuNPs, to achieve better electron transport properties and greater specific surface area and biocompatibility. Among them, the combination of GR and other nanomaterials is most frequently cited in the literature.

As shown in Figure 5B, Zhu et al.[91] utilized the catalytic effect of the g-C3N4 nanosheet (g-CNNS) on hydrogen peroxide and its high affinity for single-stranded DNA to construct a label-free electrochemical aptasensor for the detection of OTA. In the absence of OTA, specific aptamer bound to complementary DNA (cDNA) immobilized on the electrode forming double-stranded DNA that had weak affinity for g-CNNS. While with the OTA, the cDNA recovered the single-stranded state and bound g-CNNS through $\pi-\pi$ stacking, leading to the generation of a strong current by catalyzing hydrogen peroxide. The electrochemical signal was proportional to the OTA concentration in the range of 0.2−500 nM, and the LOD was 0.073 nM.

Magnetic Nanomaterials. Compared to other nanomaterials, magnetic nanomaterials (MNNs) can be combined with a variety of biomolecules to achieve their functionalization identifying and capturing targets in analytical samples quickly and efficiently, which are excellent immobilization supports of biometric elements. MNNs used in the construction of electrochemical aptasensors are usually magnetic beads (MBs) (<100 μm) and magnetic nanoparticles (MNPs) (<100 nm). At present, MBs and MNPs are used as carriers for immobilizing nucleic acid molecules for OTA detection due to their large specific surface area and unique magnetic properties, thereby enhancing the response signal and improving the sensitivity and specificity of ECBs.[92]

Rhouati et al.[93] constructed an electrochemical aptasensor based on selective capture of aptamers in mobile systems based on MNNs. Schematic representation of direct and indirect competitive format is shown in the Figure 5C. Streptavidin-coated MBs and carboxyl modified MBs provided a large number of reaction sites for OTA-biotin and aminated aptamers, respectively, and improved the analytical performances of the biosensor. The analytical principle of this method

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Table 3. Material Sensitization of OTA Electrochemical Aptasensors Based on Multiple Nanomaterials[a]

function of multiple nanomaterials									
increase of conductivity	carrier of probe (magnetic separation)	catalyst	signal tag	detection method	sensing strategy	LOD	linear range	sample	reference
	AuNPs-rGO			EIS	direct detection	30 pg/mL	0.1−20 ng/mL	red wine	102
	AuNPs-cPC			EIS	direct detection	1×10^{-8} ng/mL	1×10^{-8} to 0.1 mg/mL	soybean	103
AuNPs	CuCoPBA			EIS	direct detection	5.2 fg/mL	50 fg/mL to 10 ng/mL	fruit juice	97
AuNPs/MoS_2			MB	DPV	direct detection	0.06 nM	0.1−50 nM	wine	79
	AuNPs/cPC		MB	DPV	direct detection	5×10^{-3} pg/mL	5×10^{-6} to 5×10^{-4} ng/mL	corn	128
AuNPs/$MoSe_2$	AuNPs		MB	DPV	competitive binding	0.08 pM	0.0001−1 nM	red wine	100
AuNPs	DTN		PANI	DPV	configuration change	0.26 pg/mL	0.001−0.5 ng/mL		129
GO		nCe	nCe	CV	competitive binding	0.1 nM	0.15−180 nM	corn	130

[a]Reduced graphene oxide (rGO), carboxylic porous carbon (cPC), DNA tetrahedral nanostructure (DTN), polyaniline (PANI), nanoceria (nCe), square wave voltammetry (SWV).

is similar to the principle of direct competition and indirect competition in immunosensors. Comparing the two formats, the indirect form has a lower LOD of 0.05 μg/L. Therefore, the indirect analytical method has been widely used in OTA aptasensors.

QDs Nanomaterials. In addition to being an electrode modification material, catalyst, and signal probe, many nanomaterials also have pretty unique ECL activities.[94] QDs are nanoscale semiconductors, which will emit light of a specific frequency when suffering a certain electric field or light pressure. The ECL character of QDs is closely related to their size and chemical composition, the photochemical stability of QDs is high, and their biocompatibility is excellent. Therefore, it is an ideal luminescent material.

CdTe is a common QDs nanomaterial. As shown in Figure 5D, CdTe QDs film as an ECL donor and cyanine dye (Cy5) as an ECL receptor could generate ECL-RET resulting in a strong ECL signal. After introduction of OTA, the Cy5-modified aptamers were away from the GCE, and the ECL signal decreased significantly. Thus, the OTA concentration was inversely proportional to the ECL signal in the range of 0.0005−50 ng/mL, and the LOD is 0.17 pg/mL.[95]

Multiple Nanomaterials. Multiple nanomaterials refer to the combination of two or more different nanomaterials with similar or different properties to achieve a synergistic effect. Currently, the overwhelming majority of the electrochemical aptamer biosensors for OTA detection are based on two or more combinations of the above four single nanomaterials. The material sensitization of OTA electrochemical aptasensors based on composite or multiple material is summarized in Table 3.

Metal Matrix Nanocomposites. A series of characteristics of MNs such as small particle size, large specific surface area, and excellent biocompatibility make MNs the most prevalent nanomaterials to combine with other materials. Various metal matrix nanocomposites such as noble metal-bimetallic Prussian Blue analogs (PBAs) and noble metal-TMSs have been developed for OTA analysis. Bimetallic PBAs as a member of the metal−organic framework family are widely used in sensing research due to their easily controllable sizes and good electrocatalytic activity. However, in neutral and alkaline solutions, they exhibit poor electrical activity and low stability. Those characteristics impede their application in biosensor research.[96] Recently, a label-free electrochemical impedance aptasensor for PBAs loaded AuNPs was constructed (Figure 5F). To crown it all, loading AuNPs could improve biocompatibility and electrical conductivity of CuCoPBAs. Hence, excellent electrochemical performance was exhibited by AuNP@CuCoPBAs modified electrodes, with a wide linear range from 50 fg/mL to 10 ng/mL and a low LOD of 5.2 fg/mL. The recovery values of the OTA in spiked fruit juice samples was 93.5−104.9%.[97] As a new type of metal nanomaterial, TMSs nanomaterials have good catalytic performance, adsorption properties, and photoelectric properties. The structure of TMSs is similar to that of GR. In addition, TMSs is a semiconductor material, and its conductivity is inferior to that of GR.[98] In particular, as a carrier of noble metal nanoparticles, the combination of AuNPs-TMSs was able to exhibit high electrocatalytic activity and conductivity.[99] At present, AuNPs/MoS_2 and AuNPs/$MoSe_2$ nanocomposites have been utilized to establish ECBs for OTA detection and quantification.[79,100]

Carbon-Based Nanocomposites. Owing to some superior properties, such as excellent biocompatibility, catalysis, and ease of synthesis, CBNs are used as a carrier to support nanoparticles such as noble metals and metal oxides, which exhibit excellent catalytic and electrochemical properties.[101] Among them, CBNs combined with AuNPs have been extensively studied as an excellent signal amplification platform. When AuNPs conjugated with GR, the specific surface area and conductivity were increased, and the defects of poor solubility and easy aggregation of GR could be improved.[102,103] As shown in Figure 5E, AuNPs-rGO was utilized to modify the GCE, provide more binding site of aptamer, and enrich the electron transfer capacity of the electrode. Thus, a linear range from 0.1 to 200 ng/mL and an LOD of 0.03 ng/mL were obtained.[102]

Comparing Tables 2 and 3, it was found that electrochemical aptasensors prepared using composite or multiple materials can achieve higher sensitivity than sensors prepared using a single material. However, the preparation process of composite nanomaterials is usually more complicated than that of single nanomaterials. Therefore, the development of simpler and greener nanomaterial preparation methods is still the focus of researchers in the future.

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Table 4. Technology Sensitization of Signal-On/Off OTA Electrochemical Aptasensors[a]

technical sensitization	amplified reaction	modified electrode	signal tag	detection method	LOD	linear range	sample	reference
LAMP	on electrode		$Ru(phen)_3^{2+}$	ECL	0.3 pM	0.001−50 nM	red wine	106
	on electrode		MB	DPV	0.3 pM	0.001−50 nM	red wine	131
RCA	in solution		MB[b]	SWSV	0.065 ppt	0.1 ppt to 5 ppb	red wine	104
	in solution		Cd QDs	SWSV	0.2 pg/mL	0.5 pg/mL to 10 ng/mL	red wine	105
HRCA	on electrode		$Ru(phen)_3^{2+}$	ECL	0.02 pg/mL	0.05−500 pg/mL	corn	21
	on electrode		$Ru(phen)_3^{2+}$	ECL	8 fg/mL	0.008−50.0 pg/mL	red wine	132
endonuclease	on electrode	Hap/GE	HRP	CA	0.4 pg/mL	1.0−20 pg/mL	red wine	133
exonuclease	on electrode	APT/Fc labeled DNA/Au	Fc	DPV	1.0 pg/mL	0.005−10.0 ng/mL	wheat starch	117
	in solution		MB	DPV	0.58 pg/mL	0.001−0.5 ng/mL	wheat	112
	formation dsDNA in solution	β-CD/Au	MB	DPV	3 pg/mL	10 pg/mL ∼ 10 ng/mL	red wine	84
	in solution	APT/cDNA/ITO	$Ru(phen)_3^{2+}$	ECL	0.2 pg/mL	0.01−1.0 ng/mL	quality control corn	134

[a]Chronoamperometry (CA). [b]Represents the type of signal-off biosensor.

Technical Sensitization. Nanomaterials provide a new dimension for aptasensors construction and improve its analytical performance. Some conventional amplification technologies such as nucleic acid−based signal amplification and nuclease-based signal amplification can also play a role in OTA aptasensors. Nucleic acid−based signal amplification is a self-assembly of biomolecules based on the principle of base complementary pairing, and it includes polymerase chain reaction (PCR), rolling circle amplification (RCA),[104,105] and hyperbranched rolling circle amplification (HRCA) amplification and techniques LAMP.[106] The amplified nucleic acid can carry more electrochemical active substances to achieve signal amplification. Furthermore, on the basis of chemical synthesis and modification of low cost, good stability, and suitability for in vitro screening, mucleases have become a nonnegligible signal amplification technique in the field of electrochemical analysis. The signal amplification strategies frequently used in OTA electrochemical aptasensors are summarized in Table 4.

RCA. Because of the high requirements of temperature, equipment, and operators, although PCR technology is mature enough, those requirements have limited its application in fast, on-site detection.[21] In recent years, RCA as a burgeoning amplification technique has become an important means of detecting nucleic acids, proteins, and small molecules. RCA provided an isothermal enzymatic DNA replication technique for amplification of electrochemical signals.[107] In the traditional RCA process, a short DNA strand (linking probe) is combined with a linear DNA probe (padlock probe) in which a DNA ligase is ligated to the ends of it by the paired hybridization reaction to create a circular DNA template for rolling circle amplification. After the primer DNA is hybridized to the circular DNA template, the RCA process is activated in the presence of a mixture of nucleotides and a suitable DNA polymerase. During the RCA reaction, the circular DNA template will be replicated hundreds of times, resulting in a long single-stranded DNA that is complementary to the circular DNA template.[108,109] In a representative example, RCA combined with electrochemical aptasensor enables an ultrasensitive method of OTA detection. Huang et al.[104] constructed a novel signal-off electrochemical aptasensor based on the RCA reaction in the solution which had been successfully applied in the determination of OTA in wine samples. The RCA primer consists of two parts, one of which was the aptamer sequence for the OTA, and the other was the complement of the capture probe on the electrode surface. After the introduction of OTA, primers that originally hybridized to the RCA padlock were replaced with OTA. This resulted in the inhibition of RCA. Thus, less MB reached the Au surface, and DPV signals decreased. A linear relationship was achieved from 0.1 ng/L to 5 μg/L, and the LOD was as low as 0.065 ng/L.

HRCA. HRCA is developed on the basis of RCA and has higher electrochemical detection sensitivity. Compared with RCA, in the biosensor based on HRCA, ssDNA and dsDNA generated by the primer extension are amplified by succedent primer extension, substitution, and branching.[110] Yang et al.[21] developed a signal-on ECL biosensor based on an HRCA signal amplification strategy (Figure 6A). In the presence of OTA, aptamer combined with OTA and subsequently left the surface of Au. Thus, free ssDNA could hybridize with the padlock probe and subsequently form DNA template. Afterward, two primers initiated the HRCA process and formed a large amount of dsDNA which $Ru(phen)_3^{2+}$ could intercalate into it to generating a strong ECL signal. The LOD of this method was 0.02 pg/mL, and the linear range was from 0.05 to 500 pg/mL.

LAMP. LAMP is another isothermal amplification technique that can also be used to detect trace OTA in sample solution. It is a simple, specific, rapid, and sensitive DNA amplification method. By designing 4−6 specific primers, using the Bst DNA polymerase, the target gene can be amplified 10^9 times within 1 h.[111] As shown in Figure 6B, the aptamer was designed as an inner primer that elicited the LAMP reaction, and then the LAMP amplification products were combined with $Ru(phen)_3^{2+}$ ECL indictors. Without OTA, $Ru(phen)_3^{2+}$ was embedded in the double-stranded region of the LAMP amplicon. As the reaction proceeded, the free $Ru(phen)_3^{2+}$ concentration was reduced. Therefore, the ECL signal is inversely proportional to OTA concentration. The LOD of this method was 10 fM, and linear range was from 0.00005 to 100 nM.[106]

Nuclease. Enzyme-assisted target recovery provides an attractive option for a signal amplification strategy. Nucleases can be divided into Exo and endonuclease according to their

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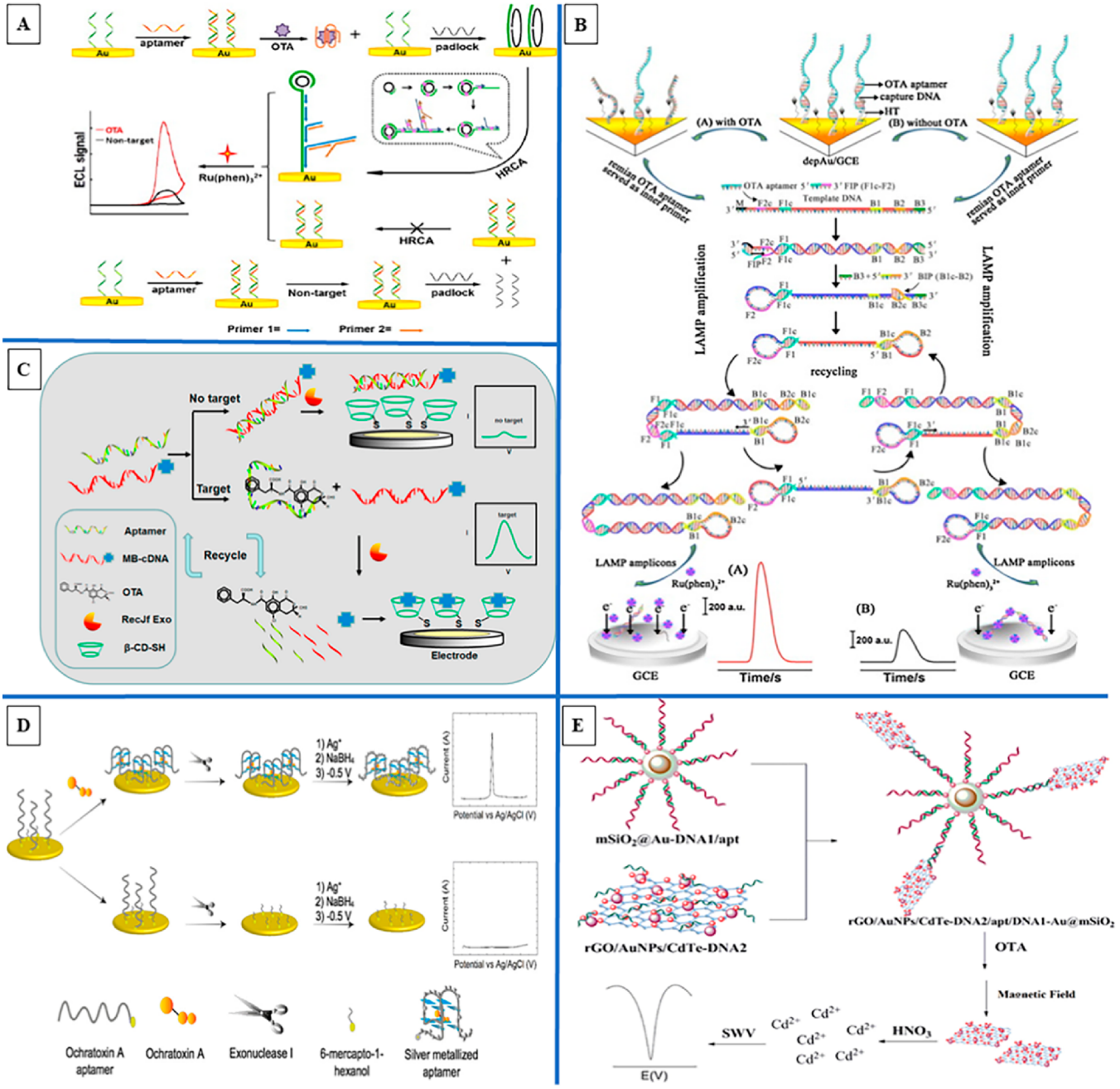

Figure 6. Schematic diagrams of technical (A−C) and combinative sensitization (D−E) of electrochemical OTA aptasensors: (A) HRCA,[21] (B) LAMP,[106] (C) Exo,[118] (D) nanomaterial@Exo,[119] (E) nanomaterial@magnetic separation technique.[122]

active site, which could identify and cleave specific nucleotide sequences or functional groups. Exo can catalyze the hydrolysis of 3,5-phosphodiester bonds from one end of the polynucleotide chain. The restriction endonuclease is an enzyme that recognizes and cleaves a specific base or base sequence. Compared with restriction endonucleases, Exo is not highly specific for sequences, so it is more flexible in biosensors for the development of enzymatic cleavage signal amplification technology. Exo III,[112] RecJf Exo,[113] Nt.BbvCI endonuclease,[114] and DNase I[115,116] are commonly used to amplify OTA aptasensor signals.

For instance, in 2011, Exocatalyzed target recovery strategy was used in ECBs to detect OTA. The Fc-labeled signal probe was immobilized on the electrode, which was complementary to the aptamer. In the presence of OTA, the aptamer which specifically bind to the OTA caused the probe reverting to single strand state. At this time, the signal probe formed a hairpin, and the Fc was attached to the surface of the electrode, resulting in an increase in the electrochemical signal. Subsequently, the aptamer that binds to the OTA was cleaved into an oligonucleotide fragment by specific Exo, and the OTA sequentially binded to the unbound aptamer on the electrode that contributed to more Fc reach the electrode surface. Thereby, a low LOD of 1.0 pg/mL was achieved.[117] Moreover, Wang et al.[118] combined β-cyclodextrin host−guest recognition with Exo signal amplification to construct a signal-on electrochemical aptasensor for OTA analysis (Figure 6C). The biggest difference from the previous method was that the MB-labeled signal probe combined with the aptamer in solution. After introduction of OTA, the signal probe and aptamer

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Table 5. Combinative Sensitization of OTA Electrochemical Aptasensors

combinative sensitization	modified electrode	signal tag	detection method	LOD	linear range	sample	reference
Nanomaterial @Nuclease	APT/DNA walker/cy5-DNA/Cd/GCE	Cds	ECL	0.012 nM	0.05−5 nM	wine, beer	78
	dsDNA/CdTe−MWCNT−CS/GCE	CdTe	ECL	0.64 pg/mL	0.001−20 ng/mL	red wine	113
	APT/SPGEs	Ag	SWV	0.7 pg/mL	1 pg/mL to 0.1 μg/mL	beer	119
Nanomaterial @Magnetic separation technique		CdTe	SWV	0.07 pg/mL	0.2 pg/mL to 4 ng/mL		122
		CdTe	SWV	5 pg/mL	10 pg/mL∼ 10 ng/mL	maize	26

returned to the single-stranded state, which was cleaved into oligonucleotide fragments by Exo at the same rate. Therefore, OTA was recycled, and the condensed signal molecules MB were recognized by the β-cyclodextrin immobilized on the Au to achieve dual signal amplification. The linear range of this method was 10 pg/mL to 10.0 ng/mL, and LOD was 3 pg/mL.

Combinative Sensitization. With the development of nanotechnology and biotechnology, the intelligent combination of nanomaterials and other signal amplification technologies has successfully constructed a series of electrochemical aptasensors. The application of combinative sensitization is summarized in Table 5.

Nanomaterial@Nuclease. Nuclease, as a simple and powerful target cyclic signal amplification method, has always been combined with nanomaterials to construct a series of aptasensor for OTA detection. For example, an OTA aptasensor employed AgNPs metallized aptamer and exonuclease I to achieve signal enhancement. As shown in Figure 6D, the combination of OTA and aptamer led to the formation of G-quadruplex, and the exonuclease I cannot play a role. Further, AgNPs metallized aptamer was formed by reduction reaction. Finally, the output difference of Ag (before and after the existence of OTA) was detected by SWV to realize OTA quantification. The shear action of exonuclease I on the unbound aptamer significantly reduced the background signal and improved the feasibility of the method. This method was verified to detect the OTA concentration in spiked beer samples and revealed good consistency with the results of gold-standard ultraperformance liquid chromatography (UPLC).[119] Under the assistance of $RecJ_f$ exonuclease, Yang et al.[113] designed an OTA ECL aptasensor. CdTe QDs composite nanofilm was covered on the electrode surface. NH_2-cDNA/aptamer-biotin duplex probes were immobilized on the film. The ECL signal of CdTe could be quenched after the alkaline phosphatase (ALP) reached the electrode surface through biotin−streptavidin (STV) combination. The presence of OTA and exonuclease-catalyzed recycling caused the biotin-aptamer to leave the electrode surface, thus inhibiting the quenching of the ECL signals induced by ALP. Therefore, the OTA concentration was proportional to the ECL signal from 0.001 to 20 ng/mL. Analogously, Wei's group designed sensitive ECL biosensors for OTA detection based on the application of the quenching ability of Cy5 to CdS QD and endonuclease-catalyzed DNA walking amplification strategy.[78]

Nanomaterial@Magnetic Separation Technique. MNNs are excellent immobilization supports of biometric elements. Meantime, because of the super-paramagnetism, the targets carried by MNNs can be quickly separated and enriched to achieve signal amplification under the external magnetic field.[120] Thus, the magnetic separation technology is also a sensitization technology. However, it is difficult to be applied to the construction of electrochemical sensors alone. Currently, combined application of the load performance and superparamagnetism of the MNNs to electrochemical aptasensors is reported more frequently.[121] In addition, some biosensors labeled with composite nanomaterials combined with magnetic separation technology have also been developed by researchers. For instance, as shown in Figure 6E, CdTe QDs combined with rGO/AuNPs forming a composite label, which could load a large number of cDNA. AuNPs functionalized SiO_2-coated Fe_3O_4 MNPs were able to load a lot of aptamers. When OTA combined with aptamer, under the action of an external magnetic field, the functionalized MNPs were gathered, and the label was disengaged. Furthermore, Cd^{2+} was released from the composite label by the dissolution of hydrochloric acid, which was detected by SWV and proportional to the concentration of OTA. Through such a dual amplified strategy of nanomaterials and magnetic separation technology, a low LOD of 0.07 pg/mL and a linear range from 0.2 pg/mL to 4 ng/mL were achieved.[122] In the same way, the magnetic separation technique provides a new idea for the simultaneous detection of multiple mycotoxins by electrochemical aptasensors. Wang et al.[26] utilized CdTe and PbS QDs coated SiO_2 as a label for simultaneous analysis dual mycotoxins. After magnetic separation and an acid-dissolution step, peak potentials of Cd^{2+} and Pb^{2+} were detected by SWV for quantitation OTA and fumonisin, respectively. This method has been successfully used to the analysis of toxins in wheat samples.

Thus, fast and in situ trace OTA detection can be achieved and satisfactory results can be obtained by combining the sensitization technologies mentioned above with structural convertible aptamers, and coupling electrochemical techniques. To be sure, there are better prospects in this field in the future.

PERSPECTIVES

According to the current research status, the development OTA electrochemical biosensors has the following trends.

(1) Developing better recognition elements to improve selectivity. At present, signal recognition elements of OTA biosensors are only antibodies, MIPs, and aptamers. Moreover, combined recognition elements did not show a huge advantage over the single identification elements in previous reports. We can be sure that a better recognition element that can overcome the defects of the existing single recognition element or the mutually reinforcing combination recognition element will be the focus of OTA ECBs.

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(2) Developing new nanomaterials and technologies to improve sensitivity. At present, the biocompatibility of nanomaterials is still the most concerneing thing in the application of nanomaterials in biosensors. Moreover, the single process of nucleic acid amplification techniques is also complex. Therefore, scientists and researchers are strongly encouraged to consider the reasonable application and combination of various nanomaterials and nucleic acid amplification techniques to improve the sensitivity of OTA biosensors.

(3) Promoting practicality by diversity research. At present, the practical application of fabricated ECBs has remained in laboratory detection, and the actual samples were tested by a standard addition method rather than batch determination OTA in real samples. Therefore, promoting the application of ECBs to real samples and implementing rapid field analysis of OTA are the main directions of researchers. Furthermore, development of a variety of ECBs for detecting OTA metabolites and construction of multi-mycotoxins simultaneous detection systems are also important in the future.

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