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Fluorescent Biosensors in the Detection of Mycotoxins: Working Principles and Applications

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Abstract. Due to their superior sensitivity and specificity, fluorescent biosensors have become highly effective tools for detecting mycotoxins. This thesis delves into the fundamental principles, material choices, and technological advancements that underpin these biosensors. Special attention is given to the role of aptamer technology. It involves synthetic nucleic acids which is known for the exceptional specificity and binding affinity. The aptamers significantly enhance biosensor performance by offering greater design versatility and more robust target recognition than conventional antibodies. Incorporating quantum dots and fluorescent dyes as signal labels further enhances the optical capabilities of these biosensors, enabling precise multicolour imaging and the ability to conduct multiplexed detection. This study underscores the essential function of fluorescent biosensors in tackling food safety challenges, particularly those bring about by mycotoxins. These sensors provide rapid and accurate detection methods for analysing complex sample matrices. The research findings presented in this work offer valuable insights into optimizing biosensor technology. This work would contribute to the advancement in food safety, environmental monitoring, and medical diagnostics. By deepening the understanding of these technologies, this work sets the stage for future innovations to protect public health further and enhance safety measures.

Keywords: Fluorescent Biosensors; Mycotoxin Detection; Aptamer Technology; Quantum Dots.

1. Introduction

Fluorescent biosensors have played a crucial role in various fields, such as biomedicine, environmental science, and food safety. Their working principle is based on the specific binding between the recognition element and the target molecule, accompanied by the change of fluorescence signal to achieve the detection of the target substance [1]. As science and technology advanced, fluorescent biosensors have evolved significantly. Initially, these sensors were based on simple models. Over time, they have developed into complex systems incorporating nanotechnology and molecular biology. This progression has enabled them to tackle a broader range of detection tasks with greater complexity and precision [2]. In mycotoxin detection, fluorescent biosensors are gradually replacing traditional detection methods as the mainstream choice due to their high sensitivity and selectivity. Mycotoxins are toxic metabolites produced by certain fungi that seriously threaten human and animal health [3]. This risk is particularly significant when storing grains and food. Traditional detection methods, such as high-performance liquid chromatography (HPLC), are known for their high accuracy [4]. However, these techniques often require complex sample pre-treatment and analytical processes. As a result, they struggle to meet the growing demand for rapid detection. In contrast, fluorescent biosensors can complete the detection in a shorter period and achieve susceptible detection of trace toxins in complex sample matrices, providing reassurance about the safety of our food and environment [4].

In this work, the working principle, material selection, and technical application of fluorescent biosensors will be discussed in detail, with a particular focus on the role of aptamer technology. Aptamers, which are synthetic small-molecule nucleic acids, are known for their exceptionally high specificity and affinity. They can recognise and bind to a wide range of target molecules, such as specific proteins, small molecules, or whole cells. Compared to traditional antibodies, aptamers are more accessible to be prepared and more flexible to be modified. This makes them particularly

advantageous in fluorescent biosensors, where they significantly enhance biosensor performance by offering greater design versatility and more robust target recognition. The sensors which incorporates quantum dots and fluorescent dyes as signal labels led to significant breakthroughs in optical performance [5].

Additionally, these designs have shown great potential for multicolour imaging and multiplexed detection applications. This research holds significant importance in advancing the application of fluorescent biosensors for mycotoxin detection. It seeks to offer more efficient and convenient detection methods by optimising and innovating existing technologies. These improvements are particularly relevant in food safety, environmental monitoring, and medical diagnosis. Ultimately, the goal is to enhance public health and social security protection.

2. Fluorescence Biosensors

2.1. Working Principle of Fluorescence Biosensors

The core of fluorescence biosensors lies in the specific binding between recognition elements and target molecules. This feature leads to changes in the fluorescence signal, thus enabling the detection of target molecules [3]. Their essential components include recognition elements, transducer elements, and signal processing systems. The recognition elements are critical parts of fluorescence biosensors, typically comprising antibodies, nucleic acids, or enzymes. These recognition elements can specifically bind to target molecules, ensuring the high selectivity of the sensors. For instance, antibodies can bind to specific antigens, and nucleic acid probes can hybridize with particular DNA or RNA sequences. Enzymes can catalyse specific biochemical reactions [4]. These specific bindings allow the recognition elements to capture the target molecules accurately. Subsequently, the transducer elements are responsible for linking the recognition elements with fluorescent labels and converting the recognition events into detectable fluorescence signals. Standard fluorescent labels include organic dyes, quantum dots, and fluorescent proteins [4]. When the recognition elements bind to target molecules, the optical properties of the fluorescent labels change such as fluorescence intensity enhancement or quenching, fluorescence lifetime alteration, etc. By detecting these changes, quantitative analysis of the target molecules can be achieved. The signal processing system is the final step in the sensing process and is responsible for detecting and analysing the fluorescence signal. Generally, fluorescence signals can be detected using microscopes, spectrophotometers, or readers. These devices can sensitively capture changes in the fluorescence signal and convert them into electrical or digital signals. Subsequently, using computers or dedicated software, the signals are processed and analysed, ultimately determining the concentration or presence of the target molecules [5].

2.2. Applications of Fluorescence Biosensors

In environmental monitoring, fluorescence biosensors are widely used to detect pollutants such as heavy metal ions, organic contaminants, and pathogenic microorganisms. These sensors can detect target substances at deficient concentrations using fluorescent labels such as quantum dots or organic dyes. For example, researchers have developed a quantum dot-based fluorescence biosensor capable of efficiently detecting lead ions, with detection limits down to the nanomolar level [6]. This high-sensitivity detection method supports early warning of environmental pollution. In medical diagnostics, fluorescence biosensors have been employed to detect various disease markers, including cancer, cardiovascular, and infectious disease markers. Fluorescent labels specifically bind to target molecules, generating significant changes in fluorescence signals [1]. Food safety is another crucial application area. Mycotoxin contamination is a global issue, severely threatening human health and food safety. The rapid detection capability of fluorescence biosensors provides an efficient solution for food safety. According to research, a fluorescence biosensor based on molecularly imprinted polymers has been developed to detect aflatoxins in grains. This sensor boasts high selectivity and sensitivity [7].

3. Aptamer Technique

The aptamer acts as a small-molecule nucleic acid fragment (DNA or RNA oligonucleotide). It is characterized by high specificity and affinity. It binds specifically to the target molecule. Compared with traditional antibodies, aptamers have the advantages of easy synthesis, flexible modification, and high stability. In mycotoxin monitoring, the introduction of aptamer technology dramatically improves the sensitivity and selectivity of sensors [8].

3.1. Quantum Dots for Signalling

Quantum Dots (QDs) are nanoparticles made of semiconductor materials, usually between 2 and 10 nm in size. Because their size is close to or smaller than the exciton border radius of the material, quantum dots exhibit a unique quantum confinement effect. This effect gives quantum dots unique optical and electronic properties, such as high fluorescence efficiency, narrow fluorescence emission peaks, and wavelength-tenable fluorescence emission. Quantum dots are widely used in fluorescent biosensors due to their high fluorescence efficiency, photostability, and tenable emission wavelength. In aptamer technology, QDs can be used as fluorescent signal labels that bind to aptamers. When the aptamer binds to the target fungal toxin, the fluorescence signal of the QDs will change, thus enabling toxin detection. For instance, researchers can use aptamers labelled with QDs that specifically bind to the toxin when detecting aflatoxin. The resulting change in fluorescence signal accurately indicates the presence and concentration of aflatoxin [9].

The fluorescence emission wavelength of a quantum dot depends on its size. Smaller quantum dots emit at shorter wavelengths and larger quantum dots emit at longer wavelengths. This characteristic makes quantum dots highly suitable for use in multicolour fluorescence imaging. Quantum dots exhibit very high photon absorption and emission efficiencies. As a result, they can produce intense and stable fluorescence signals. Compared to conventional organic dyes, quantum dots are more resistant to photobleaching. Therefore, they can easily maintain a stable fluorescence signal over extended imaging and detection times. The surface of quantum dots can be chemically modified to bind a variety of biomolecules, such as antibodies, aptamers, and nucleic acid probes, thus achieving specific biological detection [10].

3.2. Fluorescent Dyes as Signals

Fluorescent Dyes are organic molecules that absorb light at specific wavelengths and emit fluorescence at longer wavelengths. Fluorescent dyes are used in various applications from biomarkers to fluorescence microscopy imaging, all of which rely on their efficient luminescent properties. Common dyes such as Rhodamine, a widely used red fluorescent dye, have good photostability and high fluorescence quantum yield [4]. It is often used to label antibodies, nucleic acid probes, and aptamers for fluorescence microscopy imaging. In addition, fluorescein isothiocyanate (FITC), a green fluorescent dye with high fluorescence efficiency, is commonly used in immunofluorescence experiments and fluorescent labelling. Cyanine 3 (Cy3) and Cyanine 5 (Cy5) are two dyes that are widely used in fluorescent labelling. Cy3 emits yellow-green fluorescence, while Cy5 emits fluorescence at infrared wavelengths. They have good photostability and high fluorescence yield. They are widely used in DNA sequencing, gene expression analysis, and protein labelling [11].

Fluorescent dyes are widely used in DNA sequencing, gene expression analysis, and protein labelling. A wide range of fluorescent dyes is available to meet different experimental needs, covering the entire spectral range from UV to IR. Fluorescent dyes can usually bind to biomolecules through simple chemical reactions to form stable markers. They usually have high fluorescence quantum yields and can produce significant fluorescence signals even at low concentrations, facilitating detection [12].

Quantum dots and fluorescent dyes play a crucial role in fluorescent biosensors. Quantum dots are well suited for multiple detections in complex samples due to their high fluorescence efficiency, photostability, and multicolour emission capabilities. Fluorescent dyes, on the other hand, are widely used in various biomarkers and assays due to their ease of synthesis and labelling. Both fluorescent

biosensors enable these sensors to detect biomolecules and toxins at deficient concentrations. In addition, it is widely used in biomedicine, environmental monitoring, and food safety [13].

4. Mycotoxins and Detection Methods

Mycotoxin contamination is a global issue that severely threatens human health, including aflatoxins, ochratoxins, and patulin. Aflatoxins are highly toxic compounds produced by Aspergillus flavus and Aspergillus parasiticus. They are mainly found in grains contaminated in warm and humid environments. They include four types including B1, B2, G1, and G2. Aflatoxin B1 (AFB1) is the most toxic among these four types. These toxins are highly stable and complex. It can be reduced through conventional cooking. As a potent carcinogen, aflatoxin, once ingested, is metabolized in the liver, exhibiting high hepatotoxicity. During metabolism, the generated epoxide forms adducts with DNA bases, leading to DNA replication and transcription errors. Chronic exposure to low doses of aflatoxins can cause chronic poisoning, while acute poisoning can result in severe liver damage or even death. Specifically, AFB1 is metabolized in the liver through the cytochrome P450 enzyme system (CYP450), particularly CYP1A2 and CYP3A4, generating AFB1-8,9-epoxide. It is the primary active metabolite of AFB1. AFB1-8,9-epoxide can bind to DNA bases (especially guanine), forming adducts that cause DNA damage and mutations. This DNA damage is the primary mechanism of the carcinogenicity of AFB1. Additionally, AFB1 can be metabolised into AFQ1 (Aflatoxin Q1) and AFM1 (Aflatoxin M1), along with other metabolites. Although these metabolites are less toxic than AFB1, they still exhibit toxicity in the liver, milk, and other tissues. Besides carcinogenicity, aflatoxins can inhibit T cell proliferation and activation, reducing immunoglobulin production. Besides, it also leads to immunosuppression, impaired nutrient absorption, and stunted growth [14].

Mycotoxins can induce oxidative stress responses, damaging cellular structures and functions. Ochratoxins produce reactive oxygen species (ROS), which can damage cell membranes, proteins, and DNA, leading to apoptosis and necrosis. Ochratoxin A (OTA) has been proven to induce oxidative stress responses in kidney cells, resulting in kidney damage [15]. Mycotoxins also interfere with signal transduction pathways, affecting normal cellular functions. For instance, patulin inhibits the activity of intracellular signalling molecules, hindering cell proliferation and differentiation. This disruption in cell cycle regulation causes cell arrest at specific stages, ultimately leading to cell death.

Aflatoxins are detected by various methods, including early thin-layer chromatography (TLC) detection methods, which are less sensitive and less accurate than high-performance liquid chromatography (HPLC). Enzyme-linked immunosorbent assay (ELISA), on the other hand, is simple, rapid, and suitable for large-scale screening [16]. Nowadays, fluorescent biosensors using fluorescent signals for detection have high sensitivity and specificity. In addition to high sensitivity and specificity, fluorescent biosensors have easy operation and intuitive results. Several studies have demonstrated its effectiveness in detecting aflatoxins using a paper-based fluorescence method with a detection limit of 0.01ng/mL. One notable study developed an aptamer-based biosensor that utilizes gold nanoparticles (AuNPs) on a paper substrate. The method achieved rapid detection of aflatoxins in a few minutes, with a highly sensitive detection limit of 0.01ng/mL. It is suitable for applications in resource-limited settings and on-site testing. This makes them usable among non-specialists in resource-limited areas, and for rapid on-site screening [17]. In addition, the versatility and flexibility of fluorescent biosensors give them an advantage in detecting different types of mycotoxins. The design can be customized for various target molecules by varying the recognition element. For example, a fluorescent biosensor for detecting ochratoxin, based on molecularly imprinted polymers, was developed. The sensor has good selectivity and sensitivity. Meanwhile, it can accurately detect ochratoxin in complex sample matrices [18].

5. Conclusion

This study provides insights into the application and advantages of fluorescent biosensors in mycotoxin detection. It significantly discusses how the introduction of aptamer technology further enhances the sensitivity and selectivity of the sensors. Fluorescent biosensors are essential in food safety and environmental monitoring due to their rapid and accurate detection ability. Applying aptamer technology as an innovative molecular recognition method in sensor design not only overcomes the limitations of traditional antibodies but also achieves more accurate multi-detection and imaging capabilities by combining them with advanced materials. This combination of technologies effectively detects trace toxins in complex samples.

The research in this thesis demonstrates the critical role of fluorescent biosensors in addressing the growing food safety issues in modern society. Their high efficiency and accuracy give them irreplaceable advantages in large-scale screening and rapid response. In addition, the optimization strategies and technological innovations proposed in this study provide new ideas and directions for future sensor development, especially in further improving the stability of sensors in practical applications. In summary, this work demonstrates the technological potential of fluorescent biosensors and provides a scientific basis with practical value for solving the global challenge of mycotoxin detection. The results of this research positively contribute to the development of food safety technology. It also lays a solid foundation for future scientific research and practical applications.

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