

Research Article

The Construction of Liquid Crystal / Fluorescence Dual-Signal Immunosensor Based on DNA-Antibody Conjugate

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Abstract

A liquid crystal / fluorescence dual-signal immunosensor is developed for the sensitive detection of aflatoxin B1 (AFB1). Through click chemistry reaction, DNA probe is covalently conjugated to the antibody, constructing a detection system with liquid crystal (LC)/fluorescence dual-signal output capabilities. After co-incubation of DNA-antibody (Ab) conjugate, active DNA, and AFB1, the mixture is transferred to 96-well plates that have been pre-functionalized with AFB1 antigen for competitive binding. In the presence of AFB1, the separated supernatant can induce alignment changes in LC molecules, while the DNA-Ab complexes captured on the surface of 96-well plates can activate CRISPR/Cas12a to cleavage the ssDNA reporter, generating fluorescent signals. Notably, the active DNA demonstrated dual functionality. It can not only hybridize with DNA-antibody conjugate but also activate the CRISPR/Cas12a to cleavage the ssDNA reporter, generating fluorescent signals. As AFB1 concentrations increase, the reduced capture of DNA-Ab complexes on the 96-well plates leads to an increase in DNA and DNA-antibody complexes in the supernatant. It causes the LC image change from dark to bright, while the decreased capture of activator DNA results in the decrease of fluorescence signals. Overall, this dual-signal sensor exhibits high specificity, stability, and reproducibility, with simple operation enabling rapid AFB1 detection, it has the potential of application in real samples.

Keywords

DNA-Antibody Conjugate, Aflatoxin B1, CRISPR/Cas12a, Liquid Crystal, Fluorescence

1. Introduction

To establish an immunological method for signal amplification, various methods for modifying antibodies with nucleic acids have been developed. The covalent conjugation method is widely used, which typically includes chemical crosslinking strategy and enzymatic coupling strategy [1, 2]. Chemical cross-linking strategy is frequently employed to conjugate target DNA to antibodies using either bifunctional

cross-linkers or heterobifunctional cross-linkers. Numerous chemical cross-linkers have been developed for antibody modification, with common techniques including amino-carboxy coupling, maleimide-thiol group coupling, and click chemistry [3, 4]. Amino-carboxy coupling involves the activation of carboxyl groups (e.g., glutamic acid and aspartic acid residues) in an antibody using N-hydroxysuccinimide and

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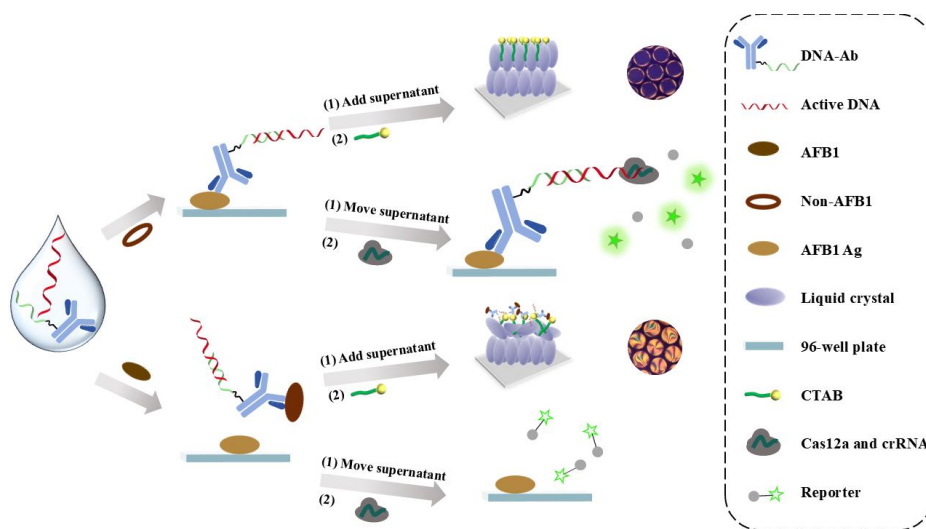
carbodiimide. Subsequently, amino-modified DNA is introduced to facilitate chemical cross-linking through this reaction. Maleimide-thiol group coupling involves the conversion of sulfhydryl groups present on the surface of the antibody into disulfide bonds through the action of a reducing agent, followed by a reaction with maleimide-modified DNA [5, 6]. The classical click chemistry reaction involves the copper-catalyzed addition of azide ($-N_3$) groups to alkyne groups. However, the presence of a copper catalyst can lead to cytotoxicity, which prompted the development of strain-promoted alkyne-azide cycloaddition (SPAAC) [7]. SPAAC does not require a catalyst and is inert to biomolecular functional groups and the biological environment. This reaction is often performed using an azide in conjunction with a dibenzocyclooctyne moiety.

Clustered regularly interspaced short palindromic repeats (CRISPR) is a powerful gene-editing technology that utilizes CRISPR-associated proteins (Cas) to create an efficient gene-editing system [8, 9]. Researchers frequently incorporate CRISPR/Cas12a into their experiments by generating fluorescent signals through this system, which amplifies signals produced by molecular recognition [10, 11]. Consequently, the introduction of CRISPR/Cas12a enhances the detection performance and sensitivity of the sensor.

Liquid crystal (LC) is a unique substance that exhibits both the fluidity of a liquid and the anisotropic properties of a crystal [12, 13]. The arrangement of liquid crystal molecules features long-range order and positional disorder,

making it highly responsive to external environmental changes [14, 15]. Consequently, this characteristic can be used to develop a sensitive LC sensor [16, 17].

Herein, a dual-mode sensor that combines LC and fluorescence for the detection of aflatoxin B1 (AFB1) using a DNA-antibody (Ab) is developed. Scheme 1 illustrates its detection principle. The diphenylcyclooctyne-tetramethylene glycol-active ester (NHS-PEG₄-DBCO) reacts with the Ab, resulting in the conjugation of a DBCO to the antibody. Subsequently, N₃-modified DNA is added, leading to the formation of the DNA-Ab conjugate after incubation. The designed active DNA serves a dual purpose: 1) it can form complementary pairs with the DNA on the antibody, allowing it to be indirectly captured by the antibody; 2) it can activate the CRISPR/Cas12a system to cleave the reporter, resulting in the generation of a fluorescent signal. The reaction solution after co-incubation of DNA-Ab, active DNA and AFB1 is transferred to AFB1 antigen (Ag) pre-modified 96-well plates for competitive binding. The AFB1 preferentially binds to DNA-Ab and less DNA-Ab is captured on the 96-well plate. Therefore, as the concentration of AFB1 increases, the active DNA and DNA-Ab in the supernatant also increases, leading to a change in the arrangement of the LC, resulting a transition from dark to bright of LC image. Furthermore, the captured DNA-Ab complexes on the 96 well plate is reduced, resulting in less active DNA available to activate CRISPR/Cas12a, thus, the fluorescence signal gradually decreases.



Scheme 1. The scheme of liquid crystal /fluorescence dual-signal sensor.

2. Materials and Methods

2.1. Materials

The nucleic acid sequences were purchased from Shanghai

Sangon Biotech Co., Ltd., China. AFB1 was bought from Shanghai Yuanye Bio-Technology Co., Ltd., China. The diphenylcyclooctyne-tetramethylene glycol-active ester (NHS-PEG₄-DBCO) was purchased by Bidepharm Co., Ltd., China. AFB1 antigen and AFB1 antibody were provided by Determine Biotechnology Co., Ltd., China.

2.2. The Modification of 96-well Plates

First, 5 $\mu\text{g/mL}$ AFB1 Ag was added to the 96-well plates and incubate at 37 $^{\circ}\text{C}$ for 2 h. After incubation, the supernatant was removed, and 0.5% gelatin-PBS blocking solution was added for incubation at 4 $^{\circ}\text{C}$ overnight.

2.3. The Conjugation of DNA-Ab

First, 1 mg/mL NHS-PEG₄-DBCO was mixed with 1 mg/mL AFB1 Ab at 30 $^{\circ}\text{C}$ for 4h. The excessive NHS-PEG₄-DBCO was filtrated using ultrafiltration tubes. Then, 1 μM N₃-modified DNA was added to react with Ab-DBCO at 37 $^{\circ}\text{C}$ for 2h.

2.4. The Detection of AFB1

Various AFB1 concentrations were mixed with DNA-Ab and active DNA at 37 $^{\circ}\text{C}$ for 30 min. Then, the reaction solution was transferred to the pre-modified 96-well plate and incubated at 37 $^{\circ}\text{C}$ for 30 min. Finally, the supernatant was collected and added to the constructed LC sensing platform (the construction method is same with previous work [18]), followed by incubation for 5 min to obtain LC image. Then, the CRISPR/Cas12a reaction solution was added into 96-well plates which have been washed three times. The fluorescence intensity at 450 nm was measured after incubating at 37 $^{\circ}\text{C}$ for 30 min.

3. Results

3.1. Feasibility of the Immunosensor

The feasibility of immunosensor is illustrated in Figure 1. First, the success of DNA-Ab coupling was confirmed through SDS-PAGE gel electrophoresis. As shown in Figure 1a, Lane 2 has a band higher than 55 kDa compared to Lane 1. The additional molecular weight is comparable to that of DNA, indicating that click chemistry was successfully executed. In addition, the recognition activities of DNA-Ab and Ab were assessed using enzyme-linked immunosorbent assay (ELISA). The antibody activity was demonstrated by comparing the OD produced by both under identical conditions, compared to Ab, DNA-Ab exhibited only a slight decrease in OD while maintaining strong recognition activity (Figure 1b). Figure 1c illustrated the feasibility of the constructed LC sensor, which displayed a bright image only when all conditions were present. Figure 1d further demonstrates the feasibility of the constructed fluorescence sensor. As shown in Figure 1d, CRISPR/Cas12a was inactive in the absence of DNA-Ab. When AFB1 was absent, the strongest fluorescent signal was obtained; In contrast, once AFB1 is present, the fluorescence signal was decreased. Therefore, the constructed fluorescence sensor is also feasible.

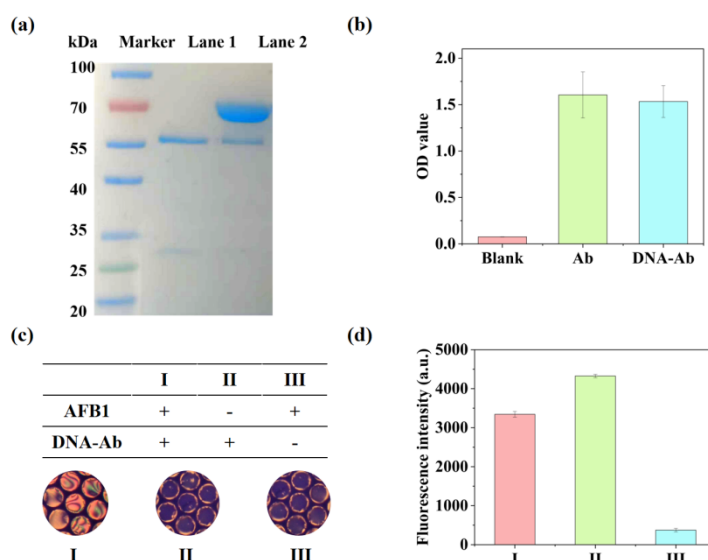


Figure 1. Feasibility of the immunosensor. (a) SDS-PAGE gel electrophoresis (Lan 1: Ab; Lane 2: DNA-Ab). (b) ELISA results for different conditions. (c) The LC image in different conditions. (d) The fluorescence intensity in different conditions.

3.2. Performance of the Immunosensor

The performance of the immunosensor was investigated with various AFB1 concentrations (0-20 ng/mL). Firstly, a series of solutions with different concentrations of AFB1 were

prepared. The corresponding LC signals and fluorescent signals were obtained using the aforementioned testing method. As illustrated in Figure 2a, the bright area coverage ratio (Br) value of the LC gradually increased with rising of AFB1 concentration, reaching a full brightness at an AFB1 concentration of 20 ng/mL . As shown in Figure 2b, the fluorescence

intensity decreased with increasing concentrations of AFB1. These results demonstrated that the constructed immunosen-

sor could be used for the quantitative detection of AFB1.

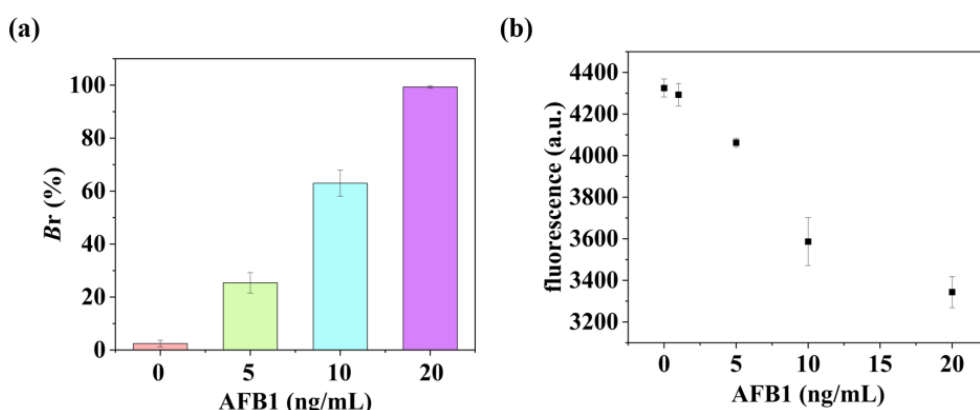


Figure 2. The detection of AFB1. (a) The Br value of the LC responding to various AFB1 concentrations. (b) The fluorescence intensity responding to various AFB1 concentrations.

4. Discussion

Compared to traditional ELISA, the developed immunosensor employs a dual-signal mode utilizing both LC and fluorescence. This approach enables semi-quantitative analysis visible to the naked eye, as well as instrumental quantification, while eliminating the need for a secondary antibody. This modification reduces reaction time and minimizes the risk of false positive signals. However, the constructed immunosensor has not yet been applied in real samples, it requires further investigation. Additionally, further experiments are required to establish a linear relationship between the LC/ fluorescence signal and AFB1 concentration.

5. Conclusions

In summary, this paper presents a dual-mode sensor utilizing LC and fluorescence for the detection of AFB1. The method employs a competitive binding approach, where AFB1 preferentially binds to the DNA-Ab complex in the presence of AFB1, resulting in the reduced capture of DNA-Ab by the 96-well plates. Consequently, as the concentration of AFB1 increases, the amount of free DNA-Ab complex in the supernatant also rises, leading the LC images have a transition from dark to bright. Additionally, the quantity of DNA-Ab complexes captured on the 96-well plate decreases, causing a gradual reduction in the fluorescence signal. Notably, the combination of DNA-Ab with CRISPR/Cas12a significantly enhances the sensitivity and selectivity of the sensor. In conclusion, the LC/fluorescence dual-mode sensor represents a sensitive, selective, convenient, and promising method for the detection of AFB1.

Abbreviations

AFB1	Aflatoxin B1
LC	Liquid Crystal
Ab	Antibody
Ag	Antigen
SPAAC	Strain-Promoted Alkyne-azide Cycloaddition
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
Cas	CRISPR-Associated Proteins
NHS-PEG ₄ -DBCO	Diphenylcyclooctyne-Tetramethylene Glycol-Active Ester
Br	Bright Area Coverage Ratio
ELISA	Enzyme-Linked Immunosorbent Assay

Author Contributions

Lijuan Zhu: Data curation, Formal Analysis, Investigation, Methodology, Writing-original draft

Qiongzhen Hu: Conceptualization, Resources, Writing-review & editing

Ru-Song Zhao: Supervision, Funding acquisition, Writing-review & editing

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Data Availability Statement

The data supporting the outcome of this research work has been reported in this manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

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