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Electronic Supplementary Information

All-in-one fabrication of a ratiometric electrochemical aptasensor with tetrahedral DNA nanostructure for fumonisin B1 detection

Na Dong[a], Shuda Liu[a], Yuye Li[a], Shuyun Meng[a], Yifan Liu[a], Xia Li[c], Dong Liu[a]*, Tianyan You[a, b]*

[a] *Key Laboratory of Modern Agricultural Equipment and Technology (Jiangsu University), Ministry of Education, School of Agricultural Engineering, Jiangsu University, Zhenjiang, Jiangsu 212013, China*

[b] *College of Agricultural Equipment Engineering, Henan University of Science and Technology, Luoyang, Henan 471003, China*

[c] *Department of Chemistry, Liaocheng University, Liaocheng, Shandong 252059, China*

*Corresponding author: Dong Liu E-mail: dongliu@ujs.edu.cn

Tianyan You E-mail: youty@ujs.edu.cn

MATERIALS AND METHODS

Reagents

Tris (2-carboxyethyl) phosphine hydrochloride (TCEP, ≥98%) came from Aladdin. Tris (hydroxymethyl) aminomethane (Tris) and 6-mercapto-1-hexanol (MCH, 98%) were provided by Alfa Aesar. Methylene blue (MB) and ethylenediaminetraacetic acid disodium salt (EDTA) were obtained from Adamas-beta. Hexaammineruthenium (III) chloride (RuHeX, 98%), aflatoxin B2 (AFB2, 98.0%), fumonisin B1 (FB1, 96.0%, J&K Chemicals), ochratoxin (OTA, 98.0%), aflatoxin B1 (AFB1, 98.0%), and zearalenone (ZEN, 98.0%) were purchased from J&K Chemicals. Gel green came from Nanjing Genesis Technology Co., Ltd. 50 bp DNA ladder, TAE (50×, pH=8), 6×DNA loading buffer, and agarose were prepared with Solarbio.

The FB1 aptamer was selected by Chen et al.[1] The DNA sequences used in the experiment are as follows (5'-3'):

•S1: TATCACCAGGCAGTTGACAGTGTAGCAAGCTGTAATAGATGCGAGGGTCCAATAC (55 mer)

•S2: TCAACTGCCTGGTGATAAAACGACACTACGTGGGAATCTACTATGGCGGCTCTTC (55 mer)

•S3: TTCAGACTTAGGAATGTGCTTCCCACGTAGTGTCGTTTGTATTGGACCCTCGCAT (55 mer)

•S4:

ACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAGAGCCGCCATAGTACGATC TGGATATTATTTTTGATACCCCTTTGGGGAGACAT (95 mer)

cDNA: SH-ATGTCTCCCCAAAGG-Fc (15 mer)

The part marked in red in S4 is the FB1 aptamer sequence.

Buffers used in the experiment are as follows:

• 10 mM phosphate buffer solution (PBS) containing 240 μM RuHex (pH=7.4) was used as electrolyte for densitometry;

• TE buffer (10 mM Tris, 1 mM EDTA, pH=8.0) and TM buffer (20 mM Tris, 50 mM

$MgCl_2$, pH=8.0) was used as DNA self-assembly solution;

• 0.1 M PBS (pH=7.4) was used as electrode rinse solution.

Apparatus

The multifunctional structure was prepared with Veriti Thermal Cycler (Applied Biosystems). G: Box gel imaging system (Gene Company Limited, UK) was used to capture gel electrophoresis image. Electrochemical impedance spectroscopy (EIS) was recorded using Autolab PGSTAT 302N electrochemical workstation (Metrohm, Netherlands) with a frequency range from 0.1 Hz to 100 kHz, an amplitude of 10 mV, and an open circuit potential of 0.27 V. CHI 660E electrochemical workstation (Chenhua, China) was used to perform square wave voltammetry (SWV) and chrono-coulometric (CC) measurements. The CC measurements were performed with an applied voltage range of 0.2 V – -0.5 V, a number of steps of 2, a pulse width of 0.25 s, and a sample interval of 0.002 s. The SWV measurements were performed with an applied voltage range of -0.4 V – 0.6 V, a potential increment of 0.004 V, an amplitude of 0.025 V, and a frequency of 15 Hz. Cyclic voltammetry (CV) was recorded using CHI 660E workstation. A three-electrode system, including an Ag/AgCl (sat. KCl) reference electrode, a platinum wire auxiliary electrode, and a gold working electrode (Φ=3 mm), was applied to all the electrochemical experiments.

Preparation of multifunctional structure

The multifunctional structure was synthesized via one-step annealing.[2] Complementary DNA (cDNA), S1, S2, S3, and S4 were dissolved in TE buffer to obtain 50 μM single-stranded DNA. Five strands were diluted to 10 μM with TM buffer, and then TCEP with a final concentration of 3 mM was added in above solutions. Finally, the diluted DNA strands were mixed and incubated at 95 °C for 2 min, then cooled to 4 °C within 10 min.

Characterization of multifunctional structure

A 4% agarose gel containing 1/100,000 of gel green was prepared and placed in a

mold for fixing molding. Multifunctional structure and other structures formed by four strands, three strands, two strands, and single strands were sampled into the prepared agarose gel and run at 110 V for 40 min.

Fabrication of the electrochemical aptasensor

Pretreatment of gold electrode: Gold electrode (Φ = 3 mm, AuE) was polished using alumina powder, and then ultrasonicated in ethanol and water for 30 s, respectively. AuE was electrochemically cleaned in 1 M H_2SO_4 with a potential range of -0.2 to 1.6 V and a scanning rate of 100 mV s^{-1}.

8 μL of multifunctional structure (2 μM) was incubated to the electrode surface at 4 °C overnight. 8 μL of MCH (1mM) was added onto the electrode surface at 37 °C for 1 h to block the nonspecific binding site. FB1 solutions were added to the sensing surface at 37 °C for 40 min. Finally, the electrodes were immersed in 2 μM MB solution for 2 min.

Preparation of real sample

Rice was pulverized in a food blender to obtain rice powder. 5 g of rice powder was extracted in 50 mL methanol-water (60:40, v/v) and shaken for 30 min. The mixture was centrifuged at 6000 rpm for 15 min, and the supernatant was filtered using 0.22 μm ultrafiltration membrane. FB1 standard was spiked to obtain sample solution of 100, 500, and 1000 ng mL^{-1}, respectively. The prepared solution was diluted to 1/10000 of the original for electrochemical testing. The ultra-performance liquid chromatography tandem mass spectrometry/mass spectrometry (UPLC-MS/MS) analysis was provided by Institute of Beijing Academy of Agriculture and Forestry Sciences.

Calculation of electroactive active area of the electrode after modification

The active electrode area after modification was estimated by CV measurements with the scan rates ranging from 25 to 150 mV s^{-1} (Fig. S2A). As shown in Fig. S2B, I_{pa} displayed a good linear relationship with the $v^{1/2}$, indicating a diffusion-controlled

redox process, which can be described by Randles-Sevcik equation (1),

$$I_{pa}=2.69\times10^{5}AD^{1/2}n^{3/2}v^{1/2}C \quad (1)$$

where A is the electroactive surface area (cm^2), D denotes the diffusion coefficient (7.6×10^{-6} cm^2 s^{-1}) of redox probe, n is the number of electrons transferred (1), v is the scan rate (V/s), and C is the concentration of redox probe (5 mM). The active electrode area after modification was calculated to be 9 mm^2.

Calculation of the limit of detection

The limit of detection (LoD) was determined using probabilistic method proposed by IUPAC, where the LoD (defined as C_L) is derived from X_L.[3] $X_L = X_b + k\,S_b$, whereby X_L means the minimum measurement signal that can be detected with reasonable certainty, X_b means the average value of the blank measurements, k means a numerical factor with a value of 3, and S_b means the standard deviation of the blank measurements. After 8 blank measurements, X_b is 0.17825 and S_b is 0.01073. The calculated X_L is 0.21044. The calibration equations for developed sensor is $I_{Fc}/I_{MB} = 0.180\ lgC_{FB1} + 0.268$. Thus, $C_L = 10^{(0.21044-0.268)/0.180} = 0.479$ pg mL^{-1}. In summary, LoD is 0.479 pg mL^{-1}.

SUPPLEMENTARY RESULTS

Fig. S1. AFM imaging of multifunctional structure on freshly cleaved mica.

Fig. S2. (A) CVs at electrode after modification with different scan rates from 25 to 150 mV s^{-1} using 5 mM $[Fe(CN)_6]^{3-/4-}$ as redox probe. (B) Plot of anodic peak current against square root of corresponding scan rate.

Table S1. Comparison of other methods for the determination of FB1.

Table S2. Comparison of developed aptasensor and UPLC-MS/MS for FB1 detection.

Fig. S1

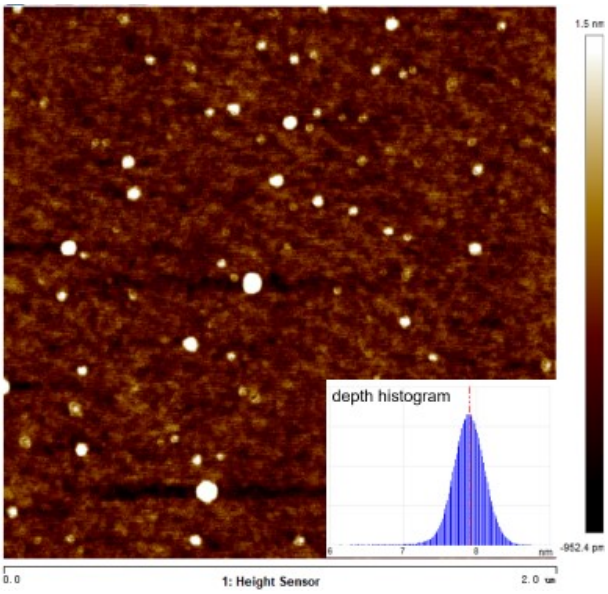

Fig. S2

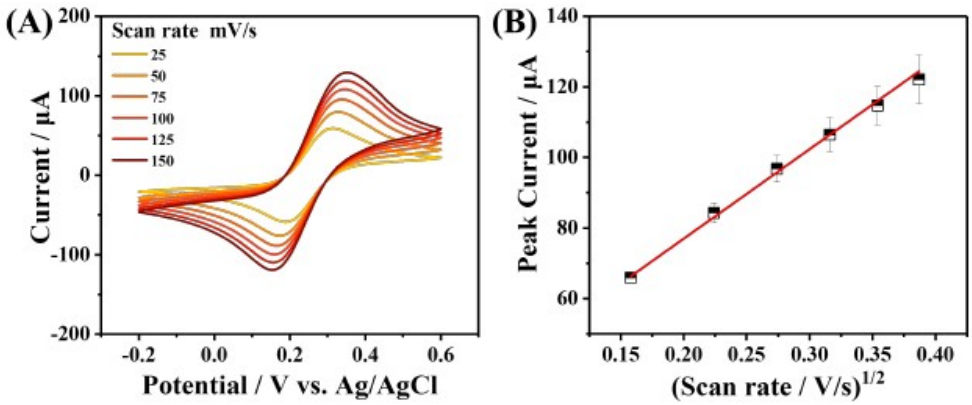
(A)
Scan rate mV/s
25
50
75
100
125
150
Current / µA
Potential / V vs. Ag/AgCl
(B)
Peak Current / µA
(Scan rate / V/s)^1/2

Table S1

Method	Linear range (pg mL^{-1})	LoD (pg mL^{-1})	Reference
LC-MS/MS	-	500000	4
ICP-MS	1000–100000	300	5
ELISA	7–7220	1	6
ICA	15000–500000	11240	7
PEC	10–1000000	4.7	8
ECL	1–100000	0.35	9
EC	1–1000000	0.15	10
SERS	62500–4000000	54860	11
FL	250–600000	60	12
Colorimetric	500–400000	150	13
EC	**0.5–500**	**0.479**	**This work**

LC-MS/MS: Liquid chromatography-tandem mass spectrometry
ICP-MS: Inductively coupled plasma mass spectrometry
ELISA: Enzyme-linked immunosorbent assay
ICA: Immunochromatographic assay
PEC: Photoelectrochemistry
ECL: Electrochemiluminescence
EC: Electrochemistry
SERS: Surface enhanced Raman scattering
FL: Fluorometry

Table S2

Sample	Spiked ($pg\ mL^{-1}$)	Developed aptasensor			UPLC-MS/MS	
		Found ($pg\ mL^{-1}$)	RSD (%)	Recovery (%)	Found ($pg\ mL^{-1}$)	Recovery (%)
Rice powder	0	7.0	4.4	/	6.5	/
	10	16.1	5.9	91.0	16.8	103
	50	55.8	4.5	97.6	60.9	109
	100	106.7	4.4	99.7	97.9	91.4

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