ACS SENSORS

pubs.acs.org/acssensors

Article

CRISPR/Cas12a-Mediated Interfacial Cleaving of Hairpin DNA Reporter for Electrochemical Nucleic Acid Sensing

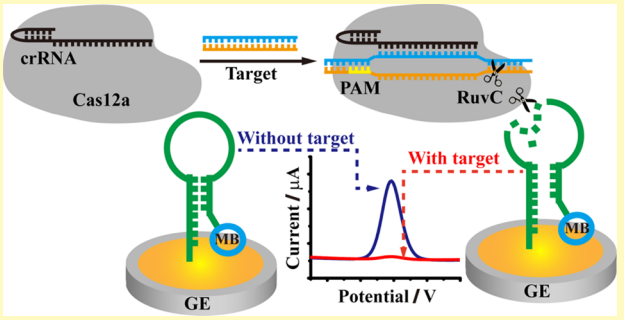
Decai Zhang,[‖] Yurong Yan,[‖] Haiying Que, Tiantian Yang, Xiaoxue Cheng, Shijia Ding, Xiuming Zhang,* and Wei Cheng*

Cite This: *ACS Sens.* 2020, 5, 557−562

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: A rapid and sensitive isothermal method is crucial for point-of-care (POC) nucleic acid testing. Recently, RNA-guided CRISPR/Cas12a proteins were discovered to exhibit target-triggered nonspecific single-stranded deoxyribonuclease (ssDNase) activity. Herein, the ssDNase cleavage capacity of the CRISPR/Cas12a system for interfacial hairpin DNA (hpDNA) and linear DNA was investigated in detailed. A novel electrochemical DNA biosensor was then developed via target-induced Cas12a cleaving interfacial hpDNA. In this strategy, the RNA-guided target DNA binding activates the robust Cas12a ssDNase activity. The immobilized hpDNA electrochemical reporters with a low surface coverage and incompact morphological structure present accessible substrates for highly efficient Cas12a cleavage, leading to a highly sensitive electrochemical DNA biosensor. Under the optimal conditions, as low as 30 pM target DNA was detected in about 60 min with 3.5 orders of magnitude dynamic range from 50 pM to 100 nM. Furthermore, the practical application ability of the established sensing method for detecting the target in complex matrices was also demonstrated. The proposed strategy enables rapid and sensitive DNA determination, providing a potential tool for POC molecular diagnostics.



KEYWORDS: *CRISPR/Cas12a, DNase, electrochemical biosensor, hairpin DNA, nucleic acid testing*

Rapid, sensitive, and selective detection of nucleic acids on a portable platform is crucial for point-of-care (POC) molecular diagnostic applications, for example, pathogen determination,[1,2] genotyping,[3] disease monitoring,[4,5] etc. Due to the limitation of complicated thermal cycling procedures in PCR-based technologies, various isothermal methods such as enzyme-assisted cycle amplification and DNA self-assembly technologies have been proposed.[6−9] However, most of the isothermal methods still rely on an initial heat-denaturation step to unwind double-stranded DNA (dsDNA) substrates, thereby limiting their applications.[10] In addition, the existing strategies often suffer from several drawbacks including long turnaround times and high operation expense as they require tedious handling procedures, harsh experimental conditions, and sophisticated devices.[11] Therefore, there is an urgent need to develop novel alternative methods to overcome the drawbacks associated with conventional strategies, especially the ones that can be implemented at a constant temperature in the whole detecting procedures and integrated compact platforms for rapid and convenient POC testing applications.

Recently, RNA-guided CRISPR-associated (Cas) proteins with different target specificities and activities, including Cas9, Cas12a, Cas13a, etc., have been exploited for sequence-specific DNA/RNA targeting and detection.[12−17] Owing to relatively easier design of guide RNAs, these programmable effectors are now being promisingly exploited for universal molecular diagnostic methodologies.[18−20] Cas12a, belonging to the class II type V-A CRISPR system,[21] can be programmed with CRISPR RNAs (crRNAs) to specifically recognize and cleave dsDNA or single-stranded DNA (ssDNA) with a RuvC catalytic pocket. Upon recognition of its target dsDNA, Cas12a can unleash single-stranded deoxyribonuclease (ssDNase) activity to indiscriminately cleave nearby ssDNAs (namely trans-cleavage).[22,23] This crRNA-programmed ssDNase activity has been utilized for detection of sequence-specific DNA in vitro.[24,25] Combined with enzymatic amplification, Cas12a-based diagnostic technologies have been proposed for testing various substances, including pathogens, single nucleotide polymorphisms (SNPs), and others.[22,26] Almost all of these methodologies utilized a linear ssDNA labeled with both a fluorophore and quencher as a substrate of activated Cas12a, which can be cleaved and release a fluorescence signal in a

Received: December 12, 2019
Accepted: February 4, 2020
Published: February 4, 2020

https://dx.doi.org/10.1021/acssensors.9b02461
ACS Sens. 2020, 5, 557−562

homogeneous environment. Although high sensitivity and specificity can be observed, such homogeneous analysis patterns rely on fluorescence readout, resulting in the requirements of bulky and expensive optical equipment, and thus limited in their ability to process point-of-care diagnostics and field analyses.

An electrochemical technique has received much attention due to easy construction, cost-effective, miniaturization, rapid response, high selectivity, and sensitivity.[27−31] More recently, a universal CRISPR/Cas12a-based electrochemical biosensor, termed E-CRISPR, has been established for DNA and protein detection.[32] However, in this method, a conventional linear ssDNA reporter is still employed to assemble on the electrode as a sensing interface, which may compromise the analytical performance of the biosensor. Two possible reasons are as follows: (i) the long distance from redox labels to the electrode surface reduces the electron transfer rate constant and results in a low electrochemical response,[33] and (ii) the steric hindrance effect on the interface may influence the interaction between Cas12a and ssDNA, producing relatively low cleavage efficiency.

Herein, a hairpin DNA (hpDNA) linked with methylene blue (MB) tag is employed to investigate the interfacial cleavage activity of Cas12a for developing an electrochemical DNA sensor. In comparison with linear DNA, the rationally designed hpDNA can shorten the electron tunneling distance between redox labels and the electrode surface and decrease the steric hindrance effect on Cas12a interfacial cleavage. Taking advantage of the hpDNA reporter and the high turnover Cas12a ssDNase activity, a dramatic electrochemical response change is achieved, producing a greatly increased analytical sensitivity with a low detection limit and a wide dynamic range. Human papillomavirus (HPV)-related DNA was chosen as the model target, which is considered to be the main cause of cervical cancer.[34] Under the optimal conditions, as low as 30 pM unamplified target DNA was detected in about 60 min with 3.5 orders of magnitude dynamic range from 50 pM to 100 nM. Moreover, the established biosensor was applied to detect target DNA in complicated DNA extracts. With excellent specificity, sensitivity, and easy to perform features, the proposed strategy promises a great potential for molecular diagnostic applications.

EXPERIMENTAL SECTION

Materials and Reagents. EnGen Lba Cas12a and 10 × NEBuffer 3.0 (1 M NaCl, 0.5 M Tris-HCl, 0.1 M $MgCl_2$, and 0.01 M DTT, pH 7.9) were obtained from New England Biolabs (Ipswich, MA, USA). HPLC-purified crRNAs, RNase inhibitor, RNase-free water, and DNA Marker were obtained from Takara Biotech. Inc. (Dalian, China). Gold View I was obtained from Solarbio Tech Co., Ltd. (Beijing, China). 6-Mercapto-1-hexanol (MCH), tris(2-carboxyethy)-phosphine hydrochloride (TCEP), and polyethylene glycol sorbitan monolaurate (Tween-20) were purchased from Sigma-Aldrich (St. Louis, USA). All DNA oligonucleotides (Table S1) were synthesized and purified from Sangon Biotech Co., Ltd. (Shanghai, China). All chemical reagents were of analytical grade, and RNase-free water was used throughout this study.

Instrumentation. All electrochemical measurements were carried out with a CHI 660D electrochemistry workstation (Shanghai Chenhua Instrument, China). A three-electrode system included a gold electrode (GE, 3 mm in diameter), a Ag/AgCl electrode, and platinum wire as working, reference, and counter electrodes, respectively. A Cary Eclipse fluorescence spectrophotometer (Agilent Technologies, Palo Alto, CA) was employed to monitor the fluorescence spectra. A NanoDrop 1000 spectrophotometer (Thermo Scientific, USA) was used to quantify DNA suspensions. The gel electrophoresis analysis was performed by an electrophoresis analyzer (Bio-Rad, USA) and imaged on a ChemiDoc XRS system (Bio-Rad, USA).

Preparation of the hpDNA-Modified Biosensing Electrode. The bare GE was freshly polished by using 0.05 μm alumina powder to obtain mirror-like smoothness followed by sonicating with ethanol and deionized water in an ultrasonic bath. The electrode was then immersed into piranha solution (H_2SO_4/H_2O_2 = 3:1) for 10 min to remove the residue and washed thoroughly with deionized water. Prior to immobilizing onto the GE surface, thiolated hpDNA was dissolved in 100 mM Tris-HCl buffer solution (containing 0.5 M NaCl, 5 mM $MgCl_2$, and 10 mM TCEP, pH 7.4). Then, 10 μL of 300 nM hpDNA was dropped onto the pretreated electrode surface and incubated at 4 °C for 12 h. After being rinsed with the washing buffer (10 mM PBS containing 0.05% (w/v) Tween-20, pH 7.4), the electrode was immersed into 100 μL of 1 mM MCH solution for 2 h to block the nonspecific sites and obtain a well-aligned DNA monolayer. Finally, after being thoroughly rinsed with the washing buffer, the electrode was allowed to be used for the following operation.

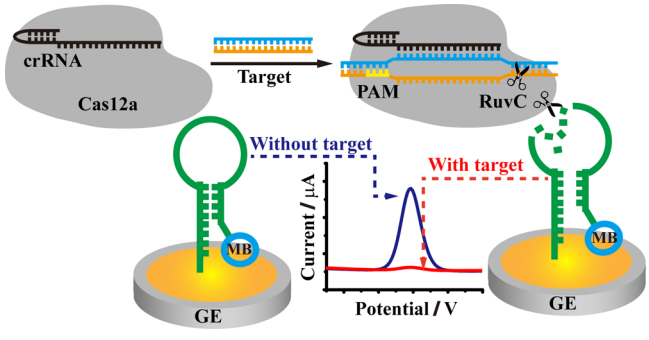
Target DNA Detection Protocol. Before the testing, the modified biosensing platform was further rinsed with the washing buffer. Subsequently, the Cas12a-mediated cleavage assay was carried out by adding 10 μL of mixture solution (50 nM Cas12a, 45 nM crRNA, 10 U RNase inhibitor, 1 × NEBuffer 3.0, and 4 μL of target DNA) and incubated for 10 min at room temperature. Afterward, the solution was dropped onto the sensor surface and incubated at 37 °C for 1 h and washed with the washing buffer. The electrochemical differential pulse voltammetry (DPV) measurements were performed in 4 mL of 10 mM PBS (pH 7.4) containing 0.5 M NaCl and 5 mM $MgCl_2$ with a modulation time of 0.05 s, an interval time of 0.017 s, a step potential of 5 mV, a modulation amplitude of 50 mV, and a potential scan from −0.5 to 0.0 V.

Gel Electrophoresis Analysis. The 12% native polyacrylamide gel was prepared to verify the ssDNase activity of Cas12a. Electrophoresis was performed in 1 × TBE buffer (2 mM EDTA and 89 mM Tris-boric acid, pH 8.3) at a 100 V constant voltage for 45 min before staining with Goldview. Finally, the gel was visualized via a gel image system.

RESULTS AND DISCUSSION

Principle of the Electrochemical Biosensing Strategy. The principle of the electrochemical biosensor based on Cas12a-mediated interfacial cleaving of hairpin DNA (hpDNA) reporter is illustrated in Scheme 1. First, crRNA, which contains a repeat sequence and a programmable target-specific sequence, can bind into Cas12a for the formation of a Cas12a-crRNA duplex. Then, the Cas12a-crRNA duplex can specifically recognize and cut target DNA based on crRNA sequence and the protospacer adjacent motif (PAM) located in

Scheme 1. Schematic Representation of the CRISPR/Cas12a-Based Electrochemical DNA Biosensing Method



https://dx.doi.org/10.1021/acssensors.9b02461
ACS Sens. 2020, 5, 557−562

the target.[15] After that, nonspecific nearby ssDNA can be digested by a Cas12a-crRNA-target DNA ternary complex. Here, an hpDNA consisting of a thiol group at the 5′ terminus and a methylene blue (MB) tag at the 3′ terminus is covalently modified to a gold electrode (GE) through S−Au bonding. In the absence of the target, the Cas12a cannot cleave the hpDNA reporter. So, the formation of a stem-loop structure brings the MB tag near the GE surface, resulting in a high redox response being detected. In the presence of the target, the ssDNase activity of Cas12a is activated, cleaving the loop region of hpDNA into short fragments and leading to the dissociation of the stem part of hpDNA. The melting temperature (T_m) of the duplex stem decreases from about 48 °C to lower than 10 °C (estimated with the IDT Oligo Analyzer, under the conditions of 10 mM Mg^{2+} and 100 mM Na^+), therefore releasing the MB from GE and decreasing the peak current. Therefore, the established electrochemical biosensor could convert per target recognition event into numerous disintegration of the hpDNA reporter on the interface for highly sensitive electrochemical DNA biosensing.

Feasibility of the Cas12a-Mediated Cleavage on the Interface. To verify the feasibility of the Cas12a-mediated cleavage effect, different reaction products were examined by polyacrylamide gel electrophoresis (PAGE). For clear electrophoretic imaging, a 32 nt linear ssDNA was used as the nonspecific substrate and incubated with a ternary complex of the Cas12a-crRNA-target. As shown in Figure 1A, the target

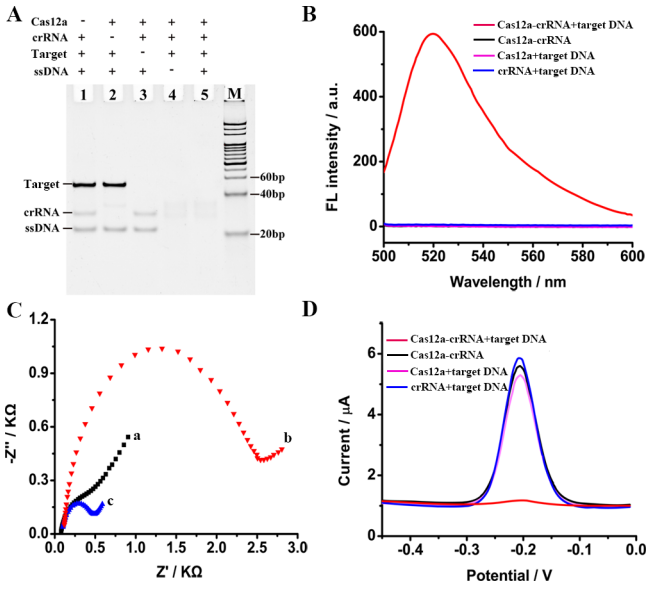


Figure 1. (A) Electrophoretic analysis of the feasibility of Cas12a-mediated cleavage assay (32 nt ssDNA as the substrate). (B) Fluorescence spectra of Cas12a-mediated cleavage fluorescence assay (λex 492 nm, λem 518 nm). (C) EIS at (a) bare GE, (b) hpDNA immobilized GE, and (c) hpDNA immobilized GE after Cas12a cleavage in 0.1 M KCl containing 5 mM $Fe(CN)_6^{3-/4-}$ (pH 6.1). (D) Typical DPV curves of MB before and after Cas12a-mediated interfacial cleaving of the hpDNA reporter.

DNA could be specifically recognized and cleaved by the Cas12a-crRNA duplex (lane 4). When the Cas12a-crRNA-target DNA complex formed, the nonspecific ssDNA was completely degraded (lane 5), indicating that the ssDNase activity of Cas12a could be successfully activated with the Cas12a-crRNA-target triplex assembly. Next, a 5 nt 5′-FAM and 3′-BHQ-labeled linear ssDNA (FQ-ssDNA) reporter was introduced into the Cas12a-mediated cleavage reaction. The result of fluorescence spectroscopy indicated that only in the formation of the Cas12a-crRNA-target DNA ternary complex could the FQ-ssDNA be efficiently cleaved and release fluorescence signals (Figure 1B). These consistent results demonstrated that, in our CRISPR/Cas12a system, the Cas12a ssDNase activity can be successfully induced with target duplex DNA for further development of the electrochemical biosensor.

In order to verify the successful fabrication of the electrochemical biosensor, electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) were employed to characterize the construction procedure. EIS was carried out with 5 mM $Fe(CN)_6^{3-/4-}$ in 0.1 M KCl solution. The semicircle diameter of the EIS curve equaled the charge-transfer resistance (Ret). As displayed in Figure 1C, the bare GE exhibited an almost straight line (curve a), reflecting excellent electrochemical conductivity. After hpDNA was immobilized onto the surface of bare GE, the Ret increased significantly (curve b). The phenomenon can be explained as that DNA has a negatively charged backbone, repulsing with the negatively charged redox probe, therefore increasing the electron transfer distance of the probe during the electrochemical reaction, which results in the higher surface impedance.[35,36] At the presence of the Cas12a-crRNA-target complex, the Cas12a ssDNase activity is activated, cutting the hpDNA off the GE surface. So, the Ret was significantly decreased in the curve c. This proved the successful assembly and target-induced cleavage of hpDNA on the sensor surface. These results were in agreement with those obtained by CV (Figure S1). Then, DPV measurements were also performed to further characterize the feasibility of the method. As displayed in Figure 1D, the high redox peak of MB at about −0.22 V was observed when the sensor was incubated without and with target DNA in the absence of the Cas12a or crRNA. In the presence of the target and Cas12a-crRNA complex, the peak current of MB dramatically decreased, which was the direct evidence of successful cleavage of hpDNA and release MB on the sensor surface.

Investigation of the Cleaving Efficiency for Interfacial hpDNA and ssDNA. To investigate the Cas12a trans-cleavage capacity for assembled hpDNA and linear DNA on the electrode surface, the DPV response was first compared in the same experimental condition. In the presence of target DNA and the Cas12a-crRNA complex, the peak current of the hpDNA reporter dramatically decreased from about 4.5 μA to approximately zero, resulting in about a 96% rate of response change (represented by ΔI%) (Figure 2A,C). However, when a linear DNA was utilized as an assembled reporter, the initial background peak current (2.5 μA) was much lower, and the sequent peak current after cleavage was still distinct (1.1 μA). The obtained ΔI% for the linear DNA reporter is only about 56%, which is similar to the results of established E-CRISPR[32] and significantly lower than that for the hpDNA reporter (Figure 2B,C). The higher initial background peak current of the hpDNA reporter is due to its higher electron transfer rate constant with closer distance between redox labels and the electrode surface than the linear DNA reporter. Meanwhile, the activated Cas12a toward a loop portion of the hpDNA exposed on the surface exhibits extremely higher cleavage efficiency owing to its different interfacial properties from the linear DNA reporter.

https://dx.doi.org/10.1021/acssensors.9b02461
ACS Sens. 2020, 5, 557−562

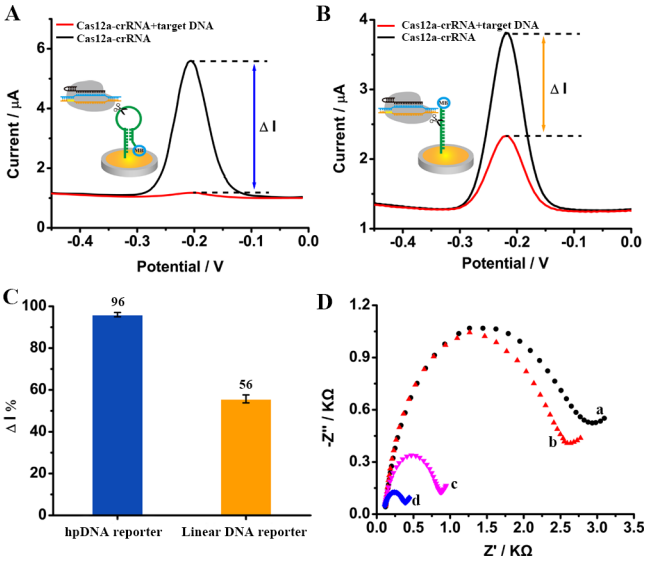


Figure 2. (A, B) Typical DPV curves of before and after Cas12a cleavage by using (A) hpDNA reporter and (B) linear DNA reporter. (C) Comparison of Δ*I*% of hpDNA reporter and linear DNA reporter. (D) EIS at (a) linear DNA modified GE, (b) hpDNA modified GE, (c) linear DNA modified GE after Cas12a cleavage, and (d) hpDNA modified GE after Cas12a cleavage in 0.1 M KCl containing 5 mM $Fe(CN)_6^{3-/4-}$ (pH 6.1). Error bars represent standard derivation obtained in three parallel experiments.

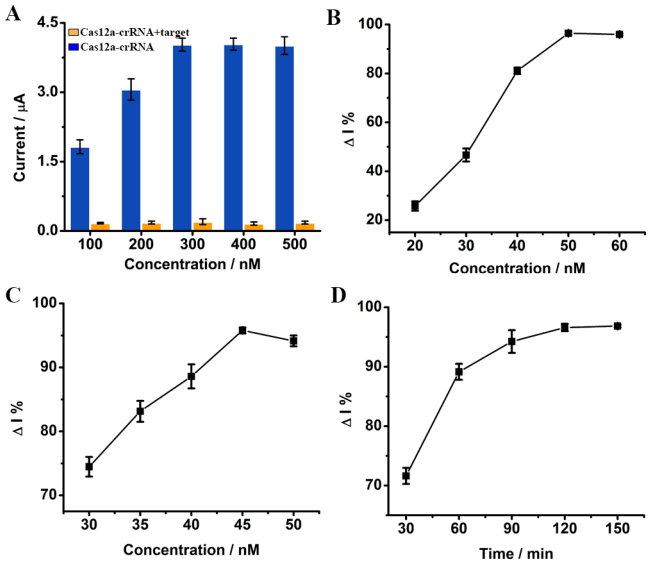


Figure 3. Optimization of the concentration of (A) hpDNA, (B) Cas12a, and (C) crRNA and (D) the reaction time. Error bars represent standard derivation obtained in three parallel experiments.

Thus, the electrochemical impedance technique was further carried out to explore the interfacial properties of the immobilized linear DNA and hpDNA. Figure 2D shows the EIS of the linear DNA and hpDNA modified GE before and after Cas12a cleavage, respectively. Before the cleavage process, the Ret of the linear DNA modified GE are higher than that of the hpDNA modified GE, indicating higher coverage and a more compact structure of the linear DNA assembly. Meanwhile, it can be clearly observed that, for the hpDNA reporter, larger variations of the Ret are recorded upon Cas12a cleavage compared to the linear DNA reporter, indicating higher cleavage efficiency toward hpDNA. These results proved that both the lower surface coverage density and incompact morphological structure contributed to a lower steric hindrance effect and higher efficiency Cas12a cleavage of the hpDNA reporter, consequentially leading to improved analytical sensitivity.

Optimization of Biosensor Preparation and Detection Conditions. Several experimental conditions were optimized via the DPV measurements for achieving optimal analytical performance. Primarily, we investigated the effect of the hpDNA packing density, which was controlled by the hpDNA incubating concentration. As can be clearly seen in Figure 3A, the peak current increased from 100 to 300 nM and then tended to a constant value. Meanwhile, after the cleavage process, the peak current dramatically decreased to approximately zero at any hpDNA concentration, indicating an extremely high Cas12a ssDNase activity and a negligible steric hindrance effect of immobilized hpDNA. Therefore, 300 nM hpDNA was chosen as the optimal concentration for the subsequent experiments. Then, the concentration of Cas12a was examined from 20 to 60 nM (Figure 3B). After 50 nM, the Δ*I*% reached a plateau, so 50 nM was chosen as the appropriate concentration. The concentration of crRNA was another important factor. The Δ*I*% increased until the concentration of crRNA exceeded 45 nM, which was then selected as the optimal concentration (Figure 3C). In addition, as the cleavage reaction time increases, the Δ*I*% improved as well (Figure 3D), indicating that the trans-cleavage mechanism is an ongoing reaction, and the amount of cleaved hpDNA is a time-dependent progress. Considering the timeliness of the method, 60 min was selected as the appropriate reaction time.

Analytical Performance of the Sensor. Under optimized experimental parameters, the dynamic range and sensitivity of the constructed biosensor was assessed. As depicted in Figure 4A, the DPV signal exhibited a gradual decrease in the range from 50 pM to 100 nM. The Δ*I*% versus the logarithm of target DNA concentration showed a good correlation in the investigated concentration range (Figure 4A, inset). The regression equation is $\Delta I\% = 0.292 \lg C + 0.476$ ($R^2 = 0.982$). The LOD was calculated to be 30 pM (3σ rule). In comparison with the linear DNA reporter-based E-CRISPR, our established biosensor shows an improved detection limit and broadened linear range.

Furthermore, the specificity of the established biosensor was investigated by using HPV types 16 and 18. As indicated in Figure 4B, HPV-16 and HPV-18 with their corresponding crRNA can induce specific electrochemical responses without obvious cross-reactivity. These results demonstrate that the established detection platform is capable of testing the target DNA with high specificity. Meanwhile, due to the programmability of crRNA, the Cas12a-based electrochemical biosensor allows development of the universal biosensing platform for any other target DNA by easily changing the crRNA guide region sequence. Moreover, the reproducibility of the biosensor has been demonstrated. According to the standard sensor construction and detection process, five parallel measurements have been performed for 1 nM target DNA. A superior reproducibility has been observed with a low relative standard deviation (RSD < 5%).

Interference Assay. To further investigate the applicability and specificity of the method for detecting target DNA in complicated DNA extracts, the DPV signal was evaluated by spiking target DNA with different concentrations in 1 μg mL^{-1}

https://dx.doi.org/10.1021/acssensors.9b02461
ACS Sens. 2020, 5, 557−562

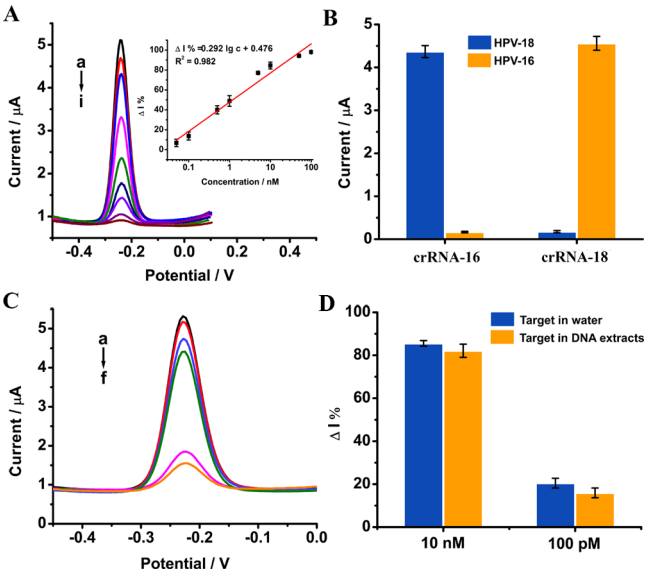


Figure 4. (A) Typical DPV curves responding to 0, 0.05, 0.1, 0.5, 1, 5, 10, 50, and 100 nM target DNA (from (a) to (i)). Inset shows the calibration curve for the Δ*I*% versus the logarithm of target DNA. (B) Specificity and universal ability of the Cas12a-based electrochemical biosensor. crRNA-16 and crRNA-18 were designed to target HPV-16 and HPV-18, respectively. (C) Typical DPV curves of 0 nM target in (a) water and (b) total DNA extracts, in (c) 0.1 nM targets in total DNA extracts and (d) water, and in (e) 10 nM targets in total DNA extracts and (f) water. (D) Comparison of Δ*I*% of different concentrations of target DNA (0.1 and 10 nM) prepared with water and DNA extracts. Error bars represent standard derivation obtained in three parallel experiments.

total DNA extracted from vaginal/anal swabs of healthy women. Figure 4C shows the comparison of the peak currents of target DNA with different concentrations in water and total DNA extracts under the same experimental condition. The DPV signals were increased a little in total DNA extracts due to the interference of the sample matrix during the target-triggered cleavage reaction. However, it can be seen that, even 100 pM target DNA in 1 μg mL^{-1} total DNA extracts, their ratio of molar concentrations was calculated to be 0.4% and was still obviously detected. The results indicated the applicability of the established system for target DNA detection in a complicated real sample.

CONCLUSIONS

In summary, a novel electrochemical sensor platform has been established for rapid and sensitive detection of nucleic acids via Cas12a-mediated interfacial cleaving of the hpDNA reporter. It was proved that both the lower surface coverage density and incompact morphological structure contributed to the lower steric hindrance effect and higher efficiency Cas12a cleavage of the hpDNA DNA reporter, consequentially leading to improved analytical sensitivity. Because of the programmability of the crRNA, the proposed biosensor will be able to detect any DNA sequences, providing a universal detection platform. Moreover, the true isothermal reaction process makes the strategy suitable for POC testing applications. In addition, the proposed strategy can also be combined with other isothermal amplification methods to construct a higher sensitivity electrochemical biosensor in the future. In a word, the proposed biosensing strategy can promise a great potential for molecular diagnostic and biochemical research applications.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acssensors.9b02461.

The oligonucleotides sequences used in this work and CV results of the immobilization and cleavage of the hpDNA reporter on the electrode (PDF)

AUTHOR INFORMATION

Corresponding Authors

Xiuming Zhang − *Department of Laboratory Diagnosis, The Third Affiliated Hospital of Shenzhen University, Shenzhen University, Shenzhen 518000, China*; Email: zxm0760@163.com

Wei Cheng − *The Center for Clinical Molecular Medical Detection, The First Affiliated Hospital of Chongqing Medical University, Chongqing 400016, China*; orcid.org/0000-0002-1921-9761; Email: chengwei@hospital.cqmu.edu.cn

Authors

Decai Zhang − *The Center for Clinical Molecular Medical Detection, The First Affiliated Hospital of Chongqing Medical University, Chongqing 400016, China; Department of Laboratory Diagnosis, The Third Affiliated Hospital of Shenzhen University, Shenzhen University, Shenzhen 518000, China*

Yurong Yan − *Key Laboratory of Clinical Laboratory Diagnostics (Ministry of Education), College of Laboratory Medicine, Chongqing Medical University, Chongqing 400016, China*

Haiying Que − *Key Laboratory of Clinical Laboratory Diagnostics (Ministry of Education), College of Laboratory Medicine, Chongqing Medical University, Chongqing 400016, China*

Tiantian Yang − *The Center for Clinical Molecular Medical Detection, The First Affiliated Hospital of Chongqing Medical University, Chongqing 400016, China*

Xiaoxue Cheng − *The Center for Clinical Molecular Medical Detection, The First Affiliated Hospital of Chongqing Medical University, Chongqing 400016, China*

Shijia Ding − *Key Laboratory of Clinical Laboratory Diagnostics (Ministry of Education), College of Laboratory Medicine, Chongqing Medical University, Chongqing 400016, China*; orcid.org/0000-0002-9183-1656

Complete contact information is available at: https://pubs.acs.org/10.1021/acssensors.9b02461

Author Contributions

‖D.Z. and Y.Y. contributed equally to this work.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was funded by the National Natural Science Foundation of China (81873972 and 81873980), The Chongqing Science Fund for Distinguished Young Scholars (cstc2019jcyjjqX0028), and the Training Program for Advanced Young Medical Personnel of Chongqing (2017HBRC003).

REFERENCES

(1) Papadakis, G.; Pantazis, A. K.; Ntogka, M.; Parasyris, K.; Theodosi, G.-I.; Kaprou, G.; Gizeli, E. 3D-printed point-of-care platform for genetic testing of infectious diseases directly in human samples using acoustic sensors and a smartphone. *ACS sens.* **2019**, *4*, 1329−1336.

(2) Hsieh, K.; Patterson, A. S.; Ferguson, B. S.; Plaxco, K. W.; Soh, H. T. Rapid, Sensitive, and Quantitative Detection of Pathogenic DNA at the Point of Care through Microfluidic Electrochemical Quantitative Loop-Mediated Isothermal Amplification. *Angew. Chem., Int. Ed.* **2012**, *51*, 4896−4900.

(3) Kong, J.; Zhu, J.; Keyser, U. F. Single molecule based SNP detection using designed DNA carriers and solid-state nanopores. *Chem. Commun.* **2017**, *53*, 436−439.

(4) Jayanthi, V. S. P. K. S. A.; Das, A. B.; Saxena, U. Recent advances in biosensor development for the detection of cancer biomarkers. *Biosens. Bioelectron.* **2017**, *91*, 15−23.

(5) Vargas, A. J.; Harris, C. C. Biomarker development in the precision medicine era: lung cancer as a case study. *Nat. Rev. Cancer* **2016**, *16*, 525−537.

(6) Feng, Q.-M.; Guo, Y.-H.; Xu, J.-J.; Chen, H.-Y. A surface-confined DNA assembly amplification strategy on DNA nanostructural scaffold for electrochemiluminescence biosensing. *Biosens. Bioelectron.* **2018**, *100*, 571−576.

(7) Du, Y.; Pothukuchy, A.; Gollihar, J. D.; Nourani, A.; Li, B.; Ellington, A. D. Coupling sensitive nucleic acid amplification with commercial pregnancy test strips. *Angew. Chem., Int. Ed.* **2017**, *56*, 992−996.

(8) Zhao, Y.; Chen, F.; Li, Q.; Wang, L.; Fan, C. Isothermal amplification of nucleic acids. *Chem. Rev.* **2015**, *115*, 12491−12545.

(9) Scheler, O.; Glynn, B.; Kurg, A. Nucleic acid detection technologies and marker molecules in bacterial diagnostics. *Expert Rev. Mol. Diagn.* **2014**, *14*, 489−500.

(10) Zhou, W.; Hu, L.; Ying, L.; Zhao, Z.; Chu, P. K.; Yu, X.-F. A CRISPR−Cas9-triggered strand displacement amplification method for ultrasensitive DNA detection. *Nat. Commun.* **2018**, *9*, 5012−5033.

(11) Furlan, I.; Domljanovic, I.; Uhd, J.; Astakhova, K. Improving the Design of Synthetic Oligonucleotide Probes by Fluorescence Melting Assay. *ChemBioChem* **2019**, *20*, 587−594.

(12) Hajian, R.; Balderston, S.; Tran, T.; deBoer, T.; Etienne, J.; Sandhu, M.; Wauford, N. A.; Chung, J.-Y.; Nokes, J.; Athaiya, M.; Paredes, J.; Peytavi, R.; Goldsmith, B.; Murthy, N.; Conboy, I. M.; Aran, K. Detection of unamplified target genes via CRISPR−Cas9 immobilized on a graphene field-effect transistor. *Nat. Biomed. Eng.* **2019**, *3*, 427−437.

(13) Huang, M.; Zhou, X.; Wang, H.; Xing, D. Clustered regularly interspaced short palindromic repeats/Cas9 triggered isothermal amplification for site-specific nucleic acid detection. *Anal. Chem.* **2018**, *90*, 2193−2200.

(14) Gootenberg, J. S.; Abudayyeh, O. O.; Lee, J. W.; Essletzbichler, P.; Dy, A. J.; Joung, J.; Verdine, V.; Donghia, N.; Daringer, N. M.; Freije, C. A.; Myhrvold, C.; Bhattacharyya, R. P.; Livny, J.; Regev, A.; Koonin, E. V.; Hung, D. T.; Sabeti, P. C.; Collins, J. J.; Zhang, F. Nucleic acid detection with CRISPR-Cas13a/C2c2. *Science* **2017**, *356*, 438−442.

(15) Murugan, K.; Babu, K.; Sundaresan, R.; Rajan, R.; Sashital, D. G. The revolution continues: newly discovered systems expand the CRISPR-Cas toolkit. *Mol. Cell* **2017**, *68*, 15−25.

(16) Nakade, S.; Yamamoto, T.; Sakuma, T. Cas9, Cpf1 and C2c1/2/3—What's next? *Bioengineered* **2017**, *8*, 265−273.

(17) Pardee, K.; Green, A. A.; Takahashi, M. K.; Braff, D.; Lambert, G.; Lee, J. W.; Ferrante, T.; Ma, D.; Donghia, N.; Fan, M.; Daringer, N. M.; Bosch, I.; Dudley, D. M.; O'Connor, D. H.; Gehrke, L.; Collins, J. J. Rapid, low-cost detection of Zika virus using programmable biomolecular components. *Cell* **2016**, *165*, 1255−1266.

(18) Li, Y.; Li, S.; Wang, J.; Liu, G. CRISPR/Cas systems towards next-generation biosensing. *Trends Biotechnol.* **2019**, *37*, 730−743.

(19) Batista, A. C.; Pacheco, L. G. C. Detecting pathogens with Zinc-Finger, TALE and CRISPR-based programmable nucleic acid binding proteins. *J. Microbiol. Methods* **2018**, *152*, 98−104.

(20) Chertow, D. S. Next-generation diagnostics with CRISPR. *Science* **2018**, *360*, 381−382.

(21) Zetsche, B.; Gootenberg, J. S.; Abudayyeh, O. O.; Slaymaker, I. M.; Makarova, K. S.; Essletzbichler, P.; Volz, S. E.; Joung, J.; van der Oost, J.; Regev, A.; Koonin, E. V.; Zhang, F. Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. *Cell* **2015**, *163*, 759−771.

(22) Chen, J. S.; Ma, E.; Harrington, L. B.; Da Costa, M.; Tian, X.; Palefsky, J. M.; Doudna, J. A. CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity. *Science* **2018**, *360*, 436−439.

(23) Li, S.-Y.; Cheng, Q.-X.; Liu, J.-K.; Nie, X.-Q.; Zhao, G.-P.; Wang, J. CRISPR-Cas12a has both cis- and trans-cleavage activities on single-stranded DNA. *Cell Res.* **2018**, *28*, 491−493.

(24) Wang, B.; Wang, R.; Wang, D.; Wu, J.; Li, J.; Wang, J.; Liu, H.; Wang, Y. Cas12aVDet: a CRISPR/Cas12a-based platform for rapid and visual nucleic acid detection. *Anal. Chem.* **2019**, *91*, 12156−12161.

(25) Li, S.-Y.; Cheng, Q.-X.; Wang, J.-M.; Li, X.-Y.; Zhang, Z.-L.; Gao, S.; Cao, R.-B.; Zhao, G.-P.; Wang, J. CRISPR-Cas12a-assisted nucleic acid detection. *Cell Discovery* **2018**, *4*, 20−24.

(26) Li, L.; Li, S.; Wu, N.; Wu, J.; Wang, G.; Zhao, G.; Wang, J. HOLMESv2: a CRISPR-Cas12b-assisted platform for nucleic acid detection and DNA methylation quantitation. *ACS Synth. Biol.* **2019**, *8*, 2228−2237.

(27) Luo, L.; Wang, Y.; Li, B.; Xu, L.; Kamau, P. M.; Zheng, J.; Yang, F.; Yang, S.; Lai, R. Molecular basis for heat desensitization of TRPV1 ion channels. *Nat. Commun.* **2019**, *10*, 2134.

(28) Que, H.; Zhang, D.; Guo, B.; Wang, T.; Wu, H.; Han, D.; Yan, Y. Label-free and ultrasensitive electrochemical biosensor for the detection of EBV-related DNA based on AgDNCs@DNA/AgNCs nanocomposites and lambda exonuclease-assisted target recycling. *Biosens. Bioelectron.* **2019**, 111610.

(29) Colozza, N.; Kehe, K.; Dionisi, G.; Popp, T.; Tsoutsoulopoulos, A.; Steinritz, D.; Moscone, D.; Arduini, F. A wearable origami-like paper-based electrochemical biosensor for sulfur mustard detection. *Biosens. Bioelectron.* **2019**, *129*, 15−23.

(30) Gu, Q.; Nanney, W.; Cao, H. H.; Wang, H.; Ye, T. Single molecule profiling of molecular recognition at a model electrochemical biosensor. *J. Am. Chem. Soc.* **2018**, *140*, 14134−14143.

(31) Yuan, R.; Ding, S.; Yan, Y.; Zhang, Y.; Zhang, Y.; Cheng, W. A facile and pragmatic electrochemical biosensing strategy for ultrasensitive detection of DNA in real sample based on defective T junction induced transcription amplification. *Biosens. Bioelectron.* **2016**, *77*, 19−25.

(32) Dai, Y.; Somoza, R. A.; Wang, L.; Welter, J. F.; Li, Y.; Caplan, A. I.; Liu, C. C. Exploring the Trans-Cleavage Activity of CRISPR-Cas12a (cpf1) for the Development of a Universal Electrochemical Biosensor. *Angew. Chem., Int. Ed.* **2019**, *58*, 17399−17405.

(33) Deng, C.; Pi, X.; Qian, P.; Chen, X.; Wu, W.; Xiang, J. High-performance ratiometric electrochemical method based on the combination of signal probe and inner reference probe in one hairpin-structured DNA. *Anal. Chem.* **2017**, *89*, 966−973.

(34) Shah, S. S.; Senapati, S.; Klacsmann, F.; Miller, D. L.; Johnson, J. J.; Chang, H.-C.; Stack, M. S. Current technologies and recent developments for screening of HPV-associated cervical and oropharyngeal cancers. *Cancers* **2016**, *8*, 85−89.

(35) Das, J.; Cederquist, K. B.; Zaragoza, A. A.; Lee, P. E.; Sargent, E. H.; Kelley, S. O. An ultrasensitive universal detector based on neutralizer displacement. *Nat. Chem.* **2012**, *4*, 642−648.

(36) Dai, Y.; Chiu, L.-Y.; Chen, Y.; Qin, S.; Wu, X.; Liu, C. C. Neutral charged immunosensor platform for protein-based biomarker analysis with enhanced sensitivity. *ACS sens.* **2019**, *4*, 161−169.

https://dx.doi.org/10.1021/acssensors.9b02461
ACS Sens. 2020, 5, 557−562