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Zi Liu	Validation; Formal analysis; Methodology; Investigation;
Lingling Xu	Formal analysis; Methodology; Investigation;
Lina Zou	Data curation; Writing - review & editing; Project administration;
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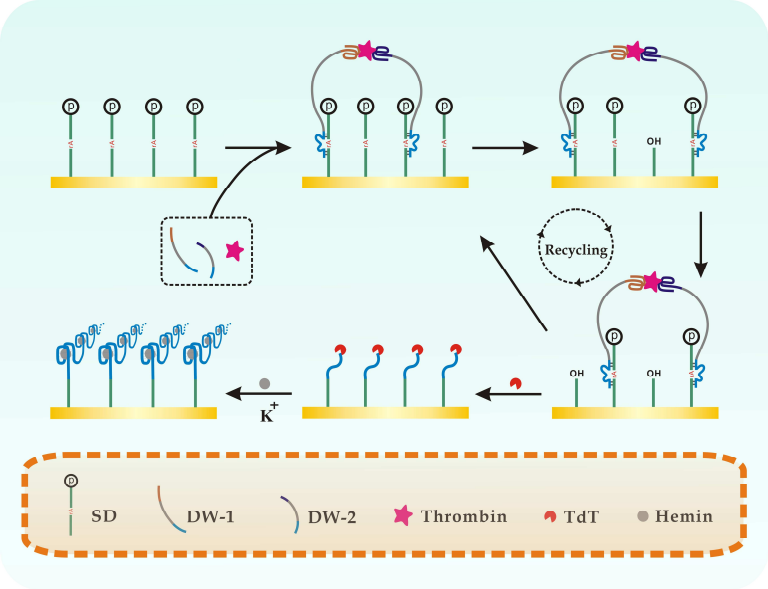

Scheme. 1 Principle of the electrochemical biosenser coupling DNAzyme-driven bipedal DNA walkers and TdT-mediated DNA elongation signal amplifications.

A "signal-on" electrochemical biosensor based on DNAzyme-driven bipedal DNA walkers and TdT-mediated cascade signal amplification strategy

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Abstract:

In this work, a dual amplified signal enhancement approach based on coupling deoxyribozyme (DNAzyme)-driven bipedal DNA walkers (BDW) and terminal deoxynucleotidyl transferase (TdT)-mediated DNA elongation signal amplifications has been developed for highly sensitive and label-free electrochemical detection of thrombin in human serums. In presence of thrombin, the BDW complex, which is comprised from the target thrombin and two DNAzyme-containing probes, can exhibit autonomous cleavage behavior on the surface of the substrate DNA (SD) modified electrode, and remove the cleaved DNA fragment from the electrode surface. Subsequently, the TdT can catalyze the elongation of the SD with free 3′-OH termini and formation of many G-quadruplex sequence replicates with the presence of 2'-deoxyaguanosine-5'-triphosphate (dGTP) and adenosine 5'-triphosphate (dATP) at a molar ratio of 6:4. These G-quadruplex sequences bind hemin and generate drastically

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amplified current response for sensitive detection of thrombin in a “signal-on” and completely label-free fashion. Under optimized conditions, the response peak current was linear with the concentration of thrombin in the range from 0.5 pM-100000 pM with detection limit of 0.31 pM. This research provides us a sustainable idea for the hyphenated multiple amplification strategies and a stable and effective method for the detection of protein biomarkers.

Keywords: DNA walker; DNAzyme; TdT; Cascade amplification; Signal-on; Label-free

1. Introduction

Detection of an ultralow amount of protein biomarkers is of great importance for early disease diagnosis. As protein biomarkers are present at trace level in human bodies, signal amplification strategies for protein detection have attracted increasing attention from bio-analytical scientists [1]. Until now, many signal amplification methods have been used in the trace protein detection, such as strand displacement amplification (SDA) [2, 3], hybridization chain reaction (HCR) [4], rolling circle amplification (RCA) [5], catalyzed hairpin assembly (CHA) [6] and nanomaterials based techniques [7-11].

Inspired by proposed DNA-based molecular machines and nano-devices, including “walkers” [12-14], “spiders” [15], “gears” [16, 17], “tweezers” [18], and so on, more and more signal amplification strategy based on the DNA-based machines (typically, DNA walkers) have been developed in the electrochemical biosensors in recent years [19, 20].

These demonstrations have included walkers driven by strand exchange reactions [6, 21], or DNAzyme/nicking enzymes-mediated DNA hydrolysis [1, 19, 20, 22]. The electrochemical biosensors based on DNAzyme/nicking enzymes-driven DNA walker mostly operated in a "signal-off" fashion. Due to the limitation of the ''signal-off'' strategy [23], a "signal-on" electrochemical assay is expecting. For example, we previously demonstrated a signal-on aptasensor based on the swing arm DNA walker strategy, in which the substrate DNA underwent Mg^{2+}-mediated DNAzyme cleavage as the walker moved autonomously [24]. However, as the swing arm-based DNA walker was tethered on the affinity ligand, it could only swing along a certain area nearby its foothold, which limits sensitivity improvement of electrochemical biosensors [6]. Furthermore, the amplification strategy based on DNA walker is single amplification actually, and the cascade of multiple amplification strategies could realize more excellent performance of electrochemical biosensor. Up to now, the cascade of multiple amplification strategy based on DNA molecular machine is still in its infancy.

In order to bypass the above disadvantages and follow the previous "signal-on" and lable-free strategy, we proposed here a "signal-on" electrochemical biosensor for sensitive determination of thrombin in human serums by coupling DNAzyme-driven bipedal DNA walkers and TdT-mediated DNA elongation signal amplifications. In the presence of thrombin, the binding of thrombin to the proximity probes (forming the BDW) simultaneously brings the tail sequences into close proximity, which increases their local concentration dramatically. It allows the DNAzyme sequences to hybridize with the

substrate nucleic acid and to trigger the DNAzyme catalyzing the hydrolysis of the substrate DNA (SD) at the ribonucleotide site [25]. Then, the 3′-termini of the cleaved SD are subjected to in situ TdT-mediated extension reaction to generate multiple G-quadruplex replicates with the help of the substrate deoxyribonucleoside triphosphates (dNTPs). TdT, a template-free DNA polymerase, could catalyze the corporation of multiple deoxyribonucleoside triphosphates to elongate the 3′-OH terminus of DNA and to consequently produce a tail [26]. Therefore, TdT-mediated DNA elongation was explored as a new approach for signal amplification applications. Especially, Liu and co-workers [27] demonstrated that the TdT could then produce randomly arrayed G-quadruplex sequences, when a dNTP pool consisting of 60% dGTP and 40% dATP was used. These G-quadruplex sequences could capture the electroactive molecule, hemin, to give the final amplified electrochemical signal, which elevated the detection sensitivity effectively. This strategy proves the possibility of integrating DNA molecular machines-based methods with diversified amplification methods to sensitively detect protein molecule.

2. Experimental section

2.1 Materials and reagents

Dimethyl sulfoxide (DMSO), hemin, 4-(2-hydroxyethyl) piperazine-1 ethanesulfonic acid sodium salt (HEPES), 6-mercapto-1-hexanol (MCH), hexaammineruthenium(III) chloride (RuHex), tris(2-carboxyethyl) phosphine hydrochloride (TCEP), Tris-HCl, and

thrombin were supplied by Sigma-Aldrich (St, Louis, MO). TdT was from Beyotime Biotechnology (Shanghai, China). Triphosphate (dGTP), deoxyadenosine triphosphate (dATP), and all oligonucleotides were purchased from Sangon Biological Engineering Technology and Services Co., Ltd. (Shanghai, China) and the sequences were summarized in Table S1. Ultrapure water (specific resistance of 18.2 MΩ·cm) was obtained from Thermo Scientific Barnstead GenPure water purification system (Thermo Fisher Scientific Co., Ltd, Shanghai, China) and used in all of the experimental processes. Human serum used for real sample analysis was supplied by the First Affiliated Hospital of our University. All buffers used in this experiment was provided in the Supplementary Material.

2.2 Preparation of the sensors.

Gold disk electrodes (AuEs, 3 mm in diameter) were cleaned by immersing in the freshly prepared piranha solution (H_2O_2:H_2SO_4 = 1:3, V/V) for 30 min, followed by rinsing with ultrapure water. After that, the AuEs were polished using slurry of 0.05 μm alumina on a standard polishing cloth. They were then subjected to a series of electrochemical cleaning steps as described previously [28].

A droplet of 5 μL SD (0.6 μM) in TNaK buffer (incubated with 10mM TCEP for 1 h) was dropped on AuE and incubated at 25 °C for 8 h. Then, the modified AuEs were incubated with 2 mM MCH for 1 h to block the unmodified sites and get the MCH/SD/AuE. At each step, the AuEs were slowly and carefully washed with TNaKT buffer and TNaK buffer, respectively.

2.3 Amplified thrombin sensing protocol.

Firstly, a mixture of 50 nM DW-1, 50 nM DW-2, and various concentrations of thrombin in TNaKMg buffer was prepared and incubated for 60 min at room temperature. 5 μL of the mixture was dropped on the MCH/SD/AuE and incubated for 60 min at room temperature. Secondly, 5 μL of the reaction mixture containing 1 mM dNTP (with a molar ratio of dGTP:dATP = 6:4) and 5 U of TdT in the TdT buffer was added to the sensor surfaces and kept at 37 °C for 1 h. Finally, the electrode was incubated with hemin (0.5 mM) in HNaKD buffer for 30 min to allow the association of hemin with the G-quadruplex sequences. Same as above, sensors were slowly and carefully washed with TNaKT buffer and TNaK buffer at each step, respectively.

2.4 Electrochemical measurement

A CHI 650A electrochemical workstation (CH Instruments, Shanghai, China) was employed for all electrochemical measurements. Cyclic voltammetry (CV), differential pulse voltammetry (DPV) and Chronocoulometric (CC) were performed by using a three-electrode arrangement: with a saturated calomel reference electrode (SCE), a platinum wire auxiliary electrode, and the modified working electrode. CV experiments were conducted in PBS containing 5 mM $[Fe(CN)_6]^{3-/4-}$ by scanning the potential between −0.2 and 0.7 V with scan rate of 50 $mV \cdot s^{-1}$. DPV measurements were performed in HNaK buffer with potential from -0.1 to -0.5 V and pulse parameters of the pulse amplitude of 50mV, the sample width of 16.7 ms and the pulse width of 25 ms. Prior to DPV measurements, HNaK buffer was purged with highly purified nitrogen for 30 min to

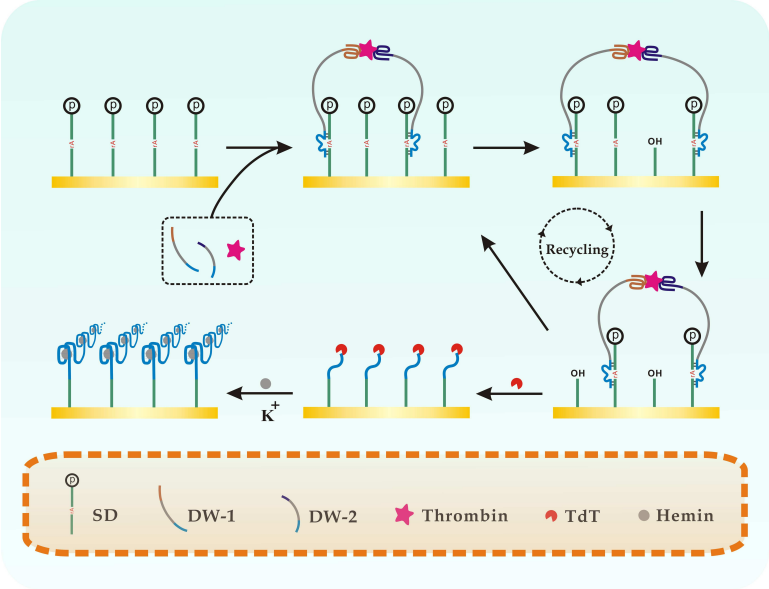
eliminate the dissolved oxygen. CC measurements were carried out at a pulse period of 500 ms and pulse width of 700 mV in 10 mM Tris-HCl buffer containing 50 μM RuHex.

3. Results and discussion

3.1 Experimental principle of the "signal-on" electrochemical biosensor

Two DNA-based affinity probes (DW-1 and DW-2) were designed with comprising three functional regions: the aptamer region (the purple sequence, Figure. S1A) for thrombin binding to form BDW, the T14 poly(T) spacer for avoiding steric hindrance during proximity-dependent hybridization, and the enzymatic sequence designed to be complementary to the SD (the blue sequence, Figure. S1A). As displayed in Scheme 1, a short oligonucleotide (SD), as the substrate of the DNAzyme, is immobilized onto a gold electrode through an alkanethiol linker at one end of the strand, while the other end is phosphorylated (Figure. S1A). In the absence of the target protein, the proximity probes cannot associate with the SD because the complementary fragment is too short to promote effective annealing (Tm < 25 □). However, in the presence of the target protein, the binding of the target molecule to the proximity probes simultaneously brings the tail sequences into close proximity enabling their local concentration increased dramatically, which allow the DNAzyme sequences to hybridize with the SD (Figure. S1B). Then, the DNAzyme effectively cleaves the SD into two short pieces, exposed the 3′-OH group. After cleavage, the single dissociated probe will quickly explore neighboring sites until it finds another intact SD to reattach and cleave, thus leading to considerable signal

amplification eventually. After that, the SD with free 3′-OH termini are further subjected to TdT-mediated DNA polymerization in a dNTP pool consisting of 40% dATP and 60% dGTP, which catalyzes repetitive addition of deoxynucleotide to elongate the exposed 3′-termini and generates massive G-quadruplex forming sequences. Upon the addition of hemin and K^+, these in situ-generated G-quadruplex sequences bind hemin to form G-quadruplex/hemin complexes on the sensor surface, and significantly amplified current response can be obtained due to electrochemical reduction of hemin to achieve highly sensitive and label-free detection of thrombin.



Scheme. 1 Principle of the electrochemical biosenser coupling DNAzyme-driven bipedal DNA walkers and TdT-mediated DNA elongation signal amplifications.

3.2 Electrochemical characterizations of the developed biosensor

To characterize the stepwise modification process of the sensing electrode, CV measurements were performed in PBS buffer containing $[Fe(CN)_6]^{3-/4-}$ (5 mM) as the redox probes. As shown in Figure 1A, the unmodified AuE gives a pair of reversible

redox peaks (curve a, Figure 1A) because of the excellent electrical conductivity of AuE. When the AuE was modified with SD (SD/AuE), a dramatic decrease of the redox currents and a significant peak separation can be observed (curve b, Figure 1A), owing to the negative charges on the DNA phosphate skeletons that prevent the redox of $[Fe(CN)_6]^{3-/4-}$ on the electrode surface. After the SD/AuE blocked with MCH (MCH/SD/AuE), the redox currents show slight increases (curve c, Figure 1A) due to the enhanced accessibility of the redox probes to the electrode interface. This is assumably ascribed to the fact that MCH could displace the physically adsorbed SD from the electrode surface and force the SD to orientate toward the electrolyte solution [37]. However, upon being incubated the mixture of DW-1, DW-2 and thrombin (DW-1&DW-2&thrombin/MCH/SD/AuE), the peak currents increase obviously (curve d, Figure. 1A). This indicates the successful DNAzyme cleavage and DNA walking processes. Moreover, subsequent TdT-mediated DNA polymerization leads to a decrease of the current peaks (curve e, Figure. 1A) because of the introduction of the elongated DNA sequences on the electrode, which significantly increases the negative charges and steric hindrance to inhibit the electron transfer. These results reveal the successful construction of the biosensors.

CC measurements are conducted to estimate DNA density on the AuE by using a cationic redox marker (RuHex). According to Cottrell expression and Faraday's law (details in Supplementary Material), the density of anchor DNA was estimated to be approximately 5.89×10^{12} molecules per cm^2 (Figure. 1B). Thus, the average area each

anchor occupies on AuE was 16.98 nm^2, and the distance between two adjacent anchors was calculated to be 5.41 nm. This density of SD was not high enough to produce steric hindrance to inhibit the reaction between BDW and SD.

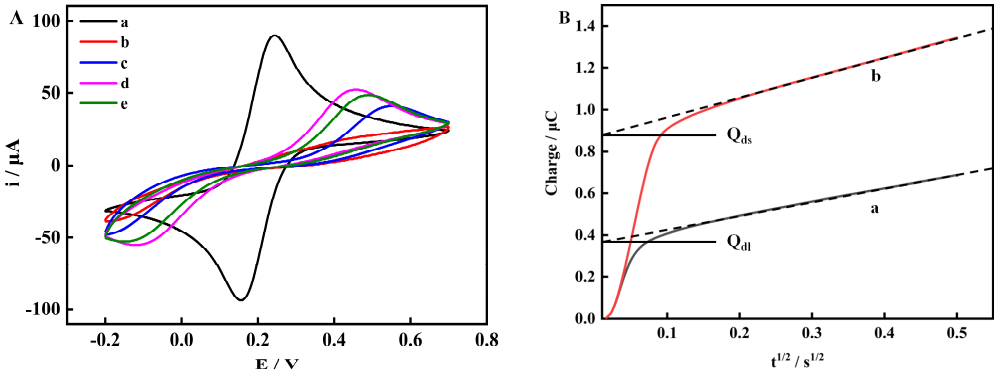


Figure. 1 (A) Cyclic voltammograms of different electrode in PBS containing 5 mM $[Fe(CN)6]^{3-/4-}$: (a) bare AuE, (b) SD/AuE, (c) MCH/SD/AuE, (d) DW-1&DW-2&thrombin/MCH/SD/AuE in 10 mM Mg^{2+}, (e) TdT/DW-1&DW-2&TB/MCH/SD/AuE.

(B) CC curves for (a) the MCH/SD/AuE and (b) the MCH/SD/AuE in 10 mM tris-HCl containing 50 μM RuHex. The CC intercept ($t^{1/2}$ =0) represents the charges of RuHex confined at the electrode surface. Qdl and Qds are the capacitive charge and the total charge containing the capacitive charge and the adsorbed reactant charge, respectively.

3.3 Feasibility of detection for thrombin with DNA walking and TdT mediated signal amplification.

To verify the feasibility of the proposed thrombin assay strategy, DPV measurements were performed with the modified electrodes. As shown in Figure. 2, when the MCH/SD/AuE were incubated respectively with the mixture of DW-1, Mg^{2+} and thrombin (curve b), the mixture of DW-2, Mg^{2+} and thrombin (curve c) and the mixture of DW-1, DW-2 and Mg^{2+} (curve d), three small DPV peaks were observed, which can be due to nonspecific absorption of hemin on the electrode surface. These data demonstrated

that the DW-1 or DW-2 could not directly initiate the enzymatic hydrolysis of SD in the presence of Mg^{2+}. However, the incubation of the sensing electrode with the mixture of DW-1, DW-2, Mg^{2+} and thrombin leads to a significant DPV current peak (curve a) at around −0.37 V (vs SCE). These observations strongly indicate that, in the presence of 10 nM thrombin, the formation of BDW initiates the enzymatic hydrolysis between the BDW and SD in the assistance of Mg^{2+}. Moreover, in the absence of Mg^{2+} and TdT (curve e and f), the DPV peak currents exhibit negligible change (vs curve b, c and d) due to the lack of DNAzyme hydrolysis or the sequence extension.

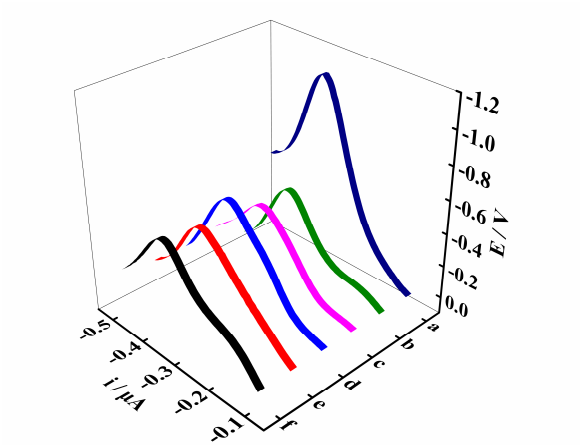


Figure. 2 DPV responses of the MCH/SD/AuE incubated with (a) DW-1, DW-2, TB and Mg^{2+}, followed by TdT-mediated DNA polymerization; (b), (c), (d), (e) and (f) are (a) in the absence of DW-1, DW-2, TB, Mg^{2+} or TDT, respectively. After that, the sensors incubated with 0.5 mM hemin for 30 min. The concentrations of DW-1, DW-2, thrombin, Mg^{2+} and TdT were 50 nM, 50 nM, 10 nM, 10 mM and 1 U/μL.

3.4 Optimizations for thrombin assay conditions

The key in this study is the binding induced cleavage. Therefore, four versions of DNAzyme used to build the BDW were tested. These DNAzymes vary by different lengths of arms-1 and arms-2 (Figure. 3A). The arm-1 of 8-17 DNAzyme ranges from 4 base pair (bp)-6 bp, and the arm-2 ranges from 7 bp-8 bp. The shorter arm-2 reduces the

background due to target-independent activity, yet a minimum length is needed for fast kinetics of the DNAzyme. As shown in Figure 3A, the DNAzymes a and b resulted in low backgrounds. Moreover, the strongest DPV response (Figure 3A) were obtained from the BDW built with DNAzymes b and c. Therefore, we chose DNAzyme b as the optimum for constructing the BDW. The density of SD on the AuE could affect the mechanical property of BDW. The maximal signal was achieved after incubating SD with 0.6 μM for 8 h at 25 °C (Figure 3B). The incubation time of BDW is another important parameter affecting the analytical performance of the biosensor. As displayed in Figure 3C, the DPV response signal increased accordingly with the extension of the incubation time and reached a plateau after 60 min, which indicated the completion of the repeated cleavage process. For TdT-mediated extension reaction (1 hour), DPV response peak current reaches a maximum value at about 1 U/μl and then no obvious change is observed (Figure. 3D).

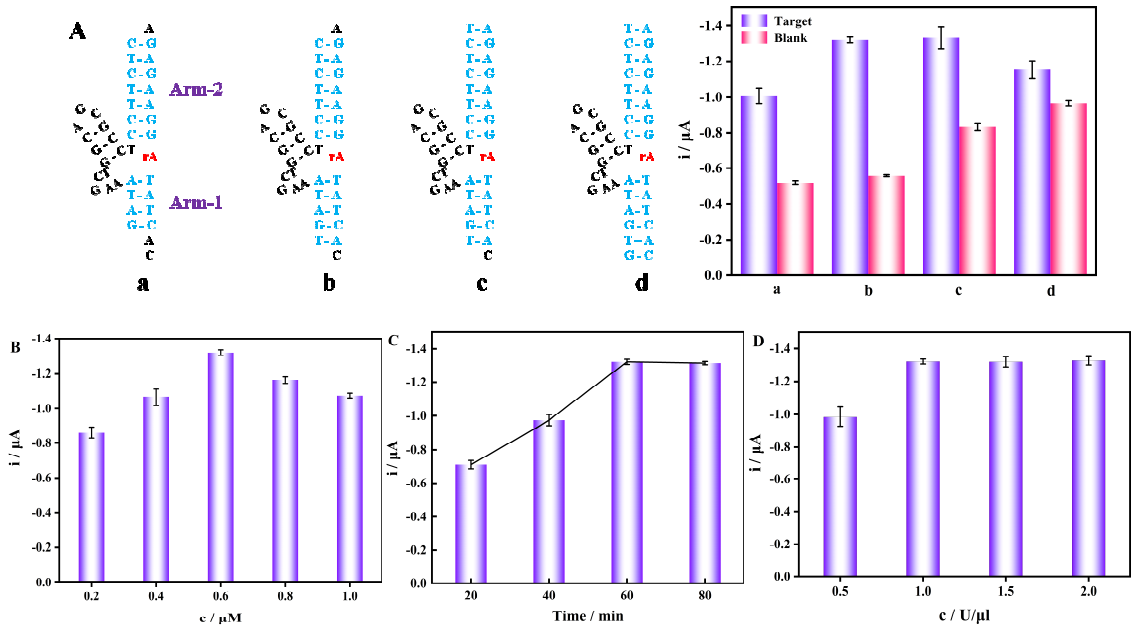


Figure. 3 Experimental optimizations for amplified electrochemical detection of thrombin: (A) four designs of walking DNA and the corresponding SD, showing (a) 7+4, (b) 7+5, (c) 8+5 and (d) 8+6 base pairing sequences. (B) the immobilization concentration of SD. (C) the BDW reaction time. (D) the concentration of TdT. Error bars represent standard deviations of three parallel experiments.

3.5 Assay performance

The performance of proposed biosensor was investigated by DPV with various concentration of thrombin under the optimal experimental conditions. As shown in Figure 4A, the DPV peak currents raise with increasing thrombin concentrations. The value of the peak currents linearly depends on the logarithm of thrombin concentration in the range from 0.5 to 100000 pM (Figure 4B). The linear equation for thrombin detection is Ip = − 0.65449 − 0.16642 log C (R^2 = 0.9929), where Ip and C represent the DPV peak current and TB concentration, respectively. The detection limit is found to be 0.31 pM according to the 3σ rule. In addition, the analytical performance comparison between the proposed thrombin assay method and other reported approaches with complex

amplification strategies is shown in Table 1.

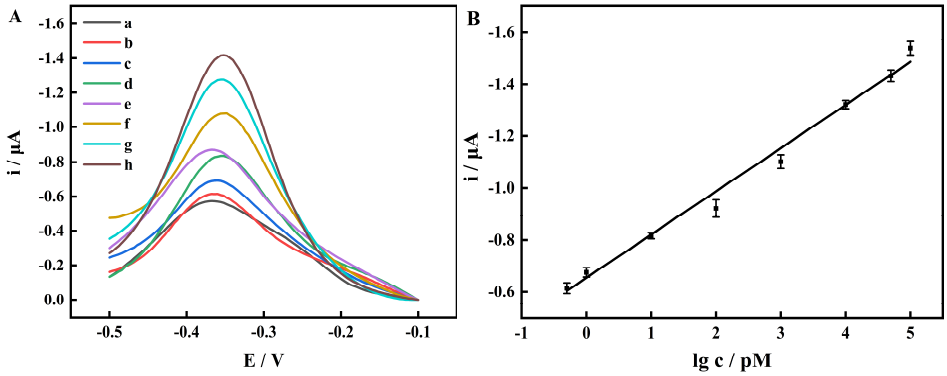


Figure. 4 (A) DPV responses of the aptasensors for different concentrations of thrombin: (a) 0 pM, (b) 0.5 pM, (c) 1 pM, (d) 10 pM, (e) 100 pM, (f) 1000 pM, (g) 10000 pM, and (h) 100000 pM. (B) Linear relationship between the DPV peak current and the logarithmic concentration of thrombin in the range from 0.5 pM to 100000 pM.

Table 1. Comparison of DNA walker strategy for thrombin detection

analytical method	amplification strategy	Linear range	Detection limit	ref
Electrochemistry	BDW	5 pM-1 nM	1.7 pM	[21]
Electrochemistry	BDW	2 pM-20 nM	0.76 pM	[22]
Electrochemistry	DNA walker	1 pM-60000 pM	0.58 pM	[24]
Electrochemistry	DNA walker	0.1 pM-10 pM	56 fM	[29]
Electrochemistry	CHA and TdT-mediated elongation	0.5 pM-10 pM	0.12 pM	[30]
Electrochemistry	DNAzyme-asisted recycle	10 pM-50 nM	5.6 pM	[31]
Photoelectrochemical	nanomaterial-based amplification	0.1 pM-8 nM	30 fM	[32]
Photoelectrochemical	CHA and nanomaterial-based amplification	50 fM-20 pM	23 fM	[33]
Electrochemiluminescence	nanomaterial-based amplification	10 fM-1 μM	0.03 fM	[34]
Electrochemiluminescence	nanomaterial-based amplification	0.01 fM-10 pM	0.01 fM	[35]
Chemiluminescence	nicking enzyme-based recycling	0 pM-1 nM	100 pM	[36]
Eletrochemistry	BDW and TdT-mediated elongation	0.5 pM-100000 pM	0.31 pM	this work

3.6 Selectivity of the electrochemical biosensor

In order to investigate the selectivity of the proposed biosensor, experiments were challenged with nonspecific proteins including CEA, Hb, INF-γ, and PSA with concentration of 100000 pM. As shown in Figure 5, no significant effect on the DPV response (vs Blank) was observed in the presence of the nontarget proteins compared with the blank test (in the absence of thrombin). Nevertheless, even with 10-fold lower thrombin (10000 pM) than the nontarget proteins, the DPV response current was much larger than the counterpart nonspecific proteins. These results demonstrate that the proposed biosensor exhibits satisfactory selectivity.

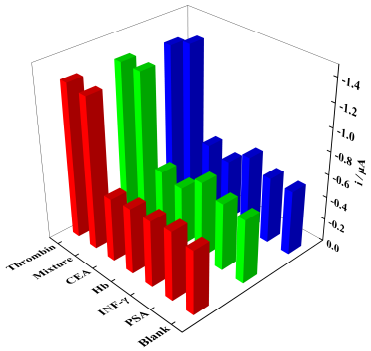


Figure. 5 Specificity of the biosensor against 100000 pM CEA, Hb, INF-γ and PSA, 10000 pM thrombin, and their mixture.

3.7. The detection of thrombin in serum samples

To evaluate the applicability of the proposed method used in real samples, 5-fold diluted healthy serum samples (from the First Affiliated Hospital of Zhengzhou University) with different concentrations of spiked TB (20, 500, and 5000 pM) were measured. The typical DPV responses corresponding to the three different measurements were displayed in Figure S2. The recoveries for TB were from 98.6 % to 104.8 % with

RSD between 2.7 % and 5.7 % (Table 2), which was acceptable for quantitative assays performed in complex real samples.

Table 2. Recovery tests for thrombin in diluted human serum samples

samples	added	found	Recovery(%)	RSD(%)
1	20 pM	21.0 pM	104.8%	5.1%
2	500 pM	500.6 pM	101.1%	5.7%
3	5000 pM	4927.6 pM	98.6%	2.7%

4. Conclusion

In conclusion, we proposed the amplification strategy based on DNA molecular machine integrated with other amplification methods on a new biosensor to sensitively detect protein molecule. The signal amplification is based on the integration of target-triggered DNA walkers and TdT-mediated polymerization formation of G-quadruplex replicates, further binding hemin and generating significantly amplified current response. The other advantages of such amplified sensor design lies in the "signal-on" fashion and completely label-free detection mode. Moreover, due to the application of the aptamers, sensor offers great potential for the detection of other proteins by using the pairs of corresponding aptamers. However, the developed biosensor needs about 13.5 h to complete the process of the thrombin detection, which limited its further application. Therefore, to develop more rapid and automatic detection strategy for the target is our effort in the future.

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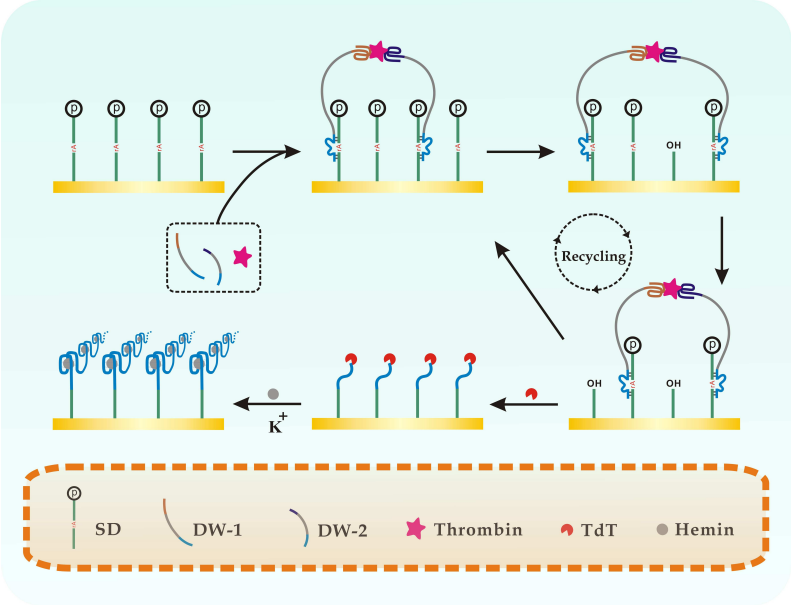

Scheme. 1 Principle of the electrochemical biosenser coupling DNAzyme-driven bipedal DNA walkers and TdT-mediated DNA elongation signal amplifications.

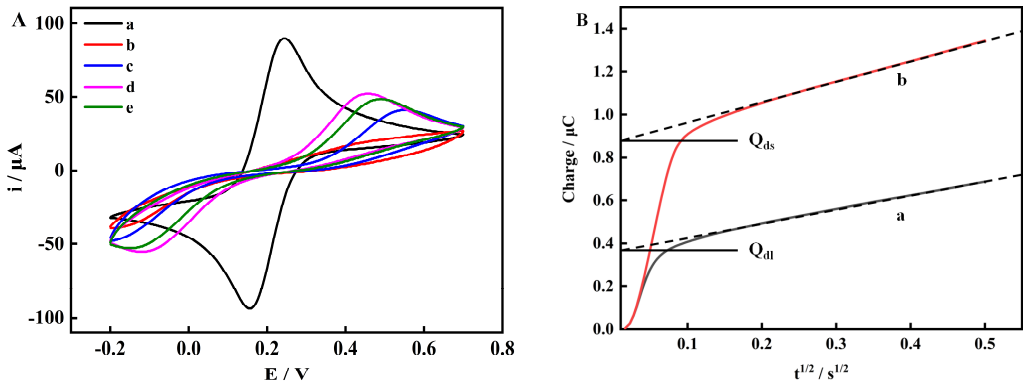

Figure. 1 (A) Cyclic voltammograms of different electrode in PBS containing 5 mM $[Fe(CN)6]^{3-/4-}$: (a) bare AuE, (b) SD/AuE, (c) MCH/SD/AuE, (d) DW-1&DW-2&thrombin/MCH/SD/AuE in 10 mM Mg^{2+}, (e) TdT/DW-1&DW-2&TB/MCH/SD/AuE.

(B) CC curves for (a) the MCH/SD/AuE and (b) the MCH/SD/AuE in 10 mM tris-HCl containing 50 μM RuHex. The CC intercept ($t^{1/2}$ =0) represents the charges of RuHex confined at the electrode surface. Qdl and Qds are the capacitive charge and the total charge containing the capacitive charge and the adsorbed reactant charge, respectively.

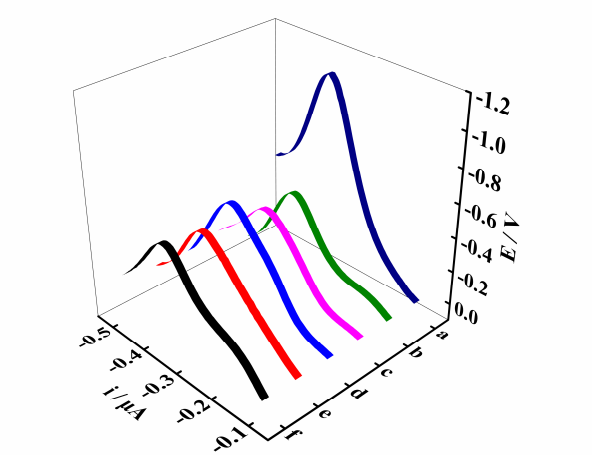

Figure. 2 DPV responses of the MCH/SD/AuE incubated with (a) DW-1, DW-2, TB and Mg^{2+}, followed by TdT-mediated DNA polymerization; (b), (c), (d), (e) and (f) are (a) in the absence of DW-1, DW-2, TB, Mg^{2+} or TDT, respectively. After that, the sensors incubated with 0.5 mM hemin for 30 min. The concentrations of DW-1, DW-2, thrombin, Mg^{2+} and TdT were 50 nM, 50 nM, 10 nM, 10 mM and 1 U/μL.

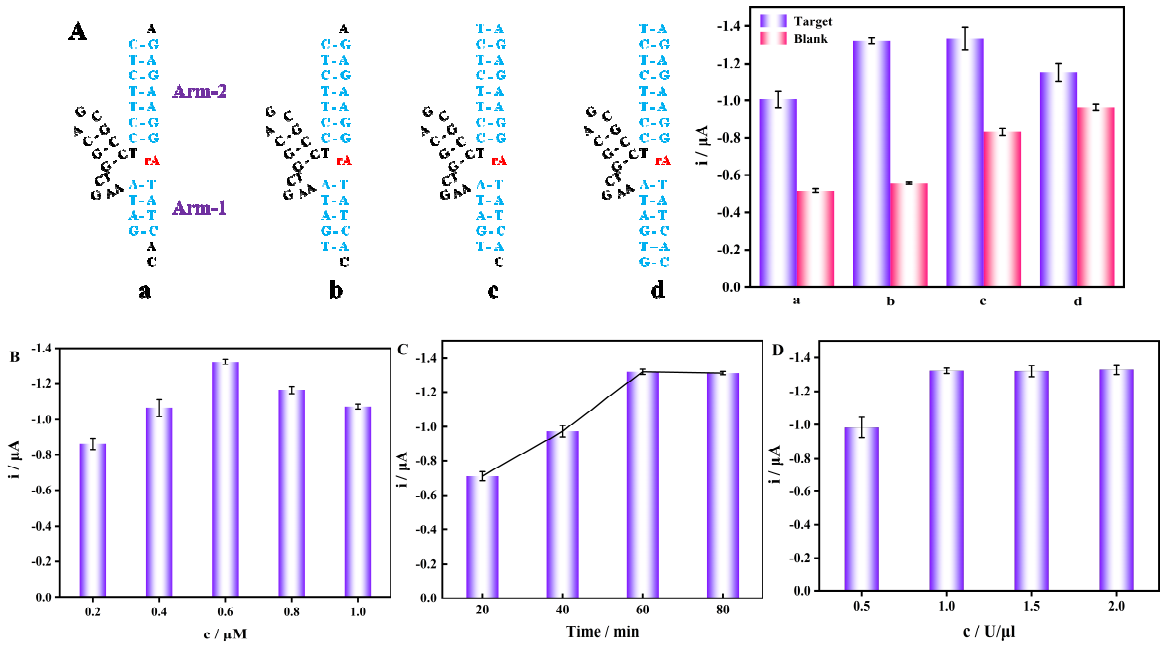

Figure. 3 Experimental optimizations for amplified electrochemical detection of thrombin: (A) four designs of walking DNA and the corresponding SD, showing (a) 7+4, (b) 7+5, (c) 8+5 and (d) 8+6 base pairing sequences. (B) the immobilization concentration of SD. (C) the BDW reaction time. (D) the concentration of TdT. Error bars represent standard deviations of three parallel experiments.

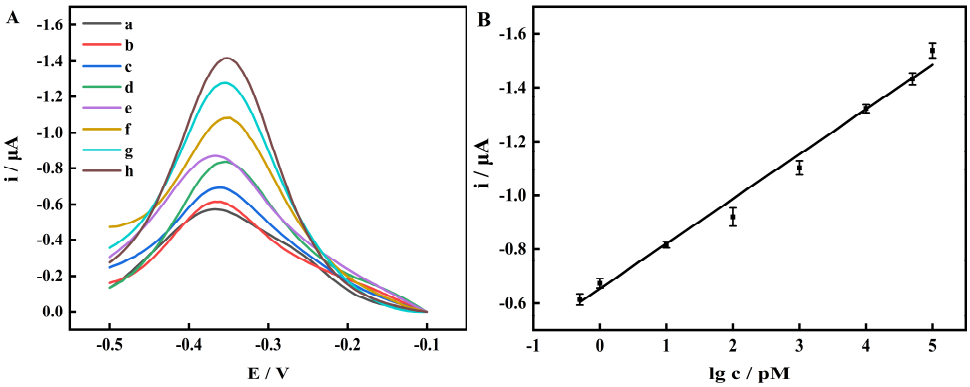

Figure. 4 (A) DPV responses of the aptasensors for different concentrations of thrombin: (a) 0 pM, (b) 0.5 pM, (c) 1 pM, (d) 10 pM, (e) 100 pM, (f) 1000 pM, (g) 10000 pM, and (h) 100000 pM. (B) Linear relationship between the DPV peak current and the logarithmic concentration of thrombin in the range from 0.5 pM to 100000 pM.

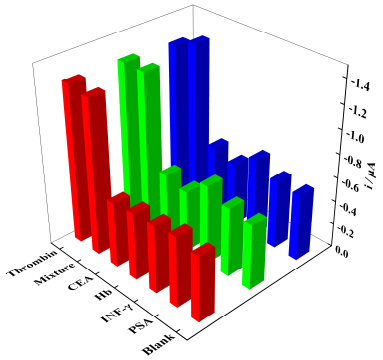

Figure. 5 Specificity of the biosensor against 100000 pM CEA, Hb, INF-γ and PSA, 10000 pM thrombin, and their mixture.

Highlights

A new electrochemical biosensor was developed based on dual cascade amplification strategy of DNAzyme-driven bipedal DNA walkers.

The developed electrochemical biosensor was completely label-free in a “signal-on” fashion.

The work proves the possibility of the strategy based on DNA molecular machine hyphenating diversified amplification methods.

The authors declare no competing financial interest.