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ARTICLE TYPE

Hybridization-induced Ag(I) dissociation from an immobilization-free and label-free haripin DNA: Toward a novel electronic monitoring platform

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A novel silver ion (Ag^+)-assisted hairpin DNA through C-Ag^+-C coordination chemistry was designed for immobilization-free and label-free electrochemical monitoring of human immunodeficiency virus (HIV) DNA on a negatively charged indium-tin oxide electrode, based on hybridization-induced dissociation of silver ion from the hairpin DNA.

Sensitive, simple, and cost-effective analysis of sequence-specific DNA has become imperative in clinical genetic diagnosis, food analysis, forensic investigation and environmental monitoring.[1] Ongoing effort has been expended in the field of assay development to simplify the assay process while preserving the essential benefits in sensitivity, robustness, broad applicability, and suitability to automation.[2] Despite some advances in this field, they have some disadvantages including complicated labels and multiple washing steps.[3] An alternative approach and strategy that does not require the probe-labeling reactions and separation steps would be advantageous.

Homogeneous assay formats are well suitable for point-of-care (POC) tests because they are simple to perform and have fast binding kinetics.[4] Typically, this methodology usually relies on devising a detection principle that is modulated and either turned on or turned off as a result of the binding reaction.[5] Unfavorably, most homogeneous assays existed until now focus on detection of fluorescence, *e.g.*, fluorescence resonance energy transfer (FRET), fluorescence polarization and time-resolved FRET.[6] In contrast, electrochemical detection has the advantages of high sensitivity, compatibility for miniaturization, easy operation and low cost, making it an attractive candidate for POC testing systems.[7] In this regard, our motivation herein is to design a novel homogeneous electrochemical assay method for detection of sequence-specific DNA based on a hairpin-structured DNA probe doped with silver ion through C-Ag^+-C coordination chemistry.

Human immunodeficiency virus (HIV) infection has evolved from a highly stigmatized disease with certain progression to acquire immunodeficiency syndrome. In this work, we design a simple and sensitive electronic monitoring platform for the in-solution detection of target DNA based on an immobilization-free and label-free hairpin DNA on a negatively charged indium-tin oxide (ITO) electrode. As a proof-of-concept study, HIV DNA (5'-ACTGCTAGAGATTTTCCACAT-3') was used as a target for DNA detection. Probe (P_1: 5'-**ACACATT**ATGTGGAA**AATCT CT**AGCAGT-3') including an underlined domain complementary to target HIV DNA and a bolded domain (form an intramolecular duplex containing two C-C mismatches) was employed as the recognition element. Scheme 1 gives the measurement process of the homogeneous electrochemical DNA sensor toward HIV DNA. The assay was carried out on a negatively charged ITO working electrode (S ≈ 3.14 mm^2) containing a platinum-wire auxiliary electrode and an Ag/AgCl reference electrode. Before each run, the negatively charged ITO electrode was prepared by sequential sonication in an Alconox solution (8.0 g of Alconox L^{-1} of water) for 15 min, propan-2-ol for 15 min, and twice in water for 15 min.[8]

Next, the Ag^+-assisted hairpin DNA *via* C-Ag^+-C coordination chemistry was prepared as follows. Briefly, 5 μL of 100 μM P_1 probe and 5 μL of 250 μM Ag^+ ion were initially mixed to 90 μL HEPES buffer [pH 7.4, 10 mM 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid + 50 mM KNO_3]. Then, the resulting mixture was denatured for 4 min at 94 °C, annealed for 5 min at 50 °C, and finally cooled to room temperature. During this process, the dopant-silver ion was sequestered into the hairpin by the C-Ag^+-C coordination chemistry. Following that, DNA hairpins (100 μL) were transferred into the above-prepared ITO detection cell. At this time, the electroactive material (Ag^+) was far away from the electrode surface, thus the electronic circuit was switched off. Upon target DNA introduction, the hybridization reaction was implemented between target DNA and hairpin DNA, which caused the disruption of the hairpin probes to release the doped silver ions. The dissociated silver ions could be captured through the negatively charged ITO electrode, thereby activating the electrical contact between silver ion and the base electrode, which resulted in the sensor circuit to switch on. Just as hybridization-induced dissociation of silver ions, the hairpin DNA switch was easily tuned by the added target DNA. By monitoring the change in the current, we could quantitatively evaluate the concentration of target DNA in the sample. In the absence of target DNA, the hairpin DNA with C-Ag^+-C pairs were homogeneously dispersed into the detection solution, hence exhibiting a weak electronic signal within the applied potentials.

As described above, the electronic signal mainly derived from the released silver ion from the Ag^+-assisted hairpin probe in the presence of target DNA. Therefore, one important precondition for the development of the homogeneous electrochemical DNA sensor was whether the negatively charged ITO electrode could exhibit high sensitivity for the detection of sliver ion. To clarify this point, different-concentration sliver ions were monitored by using the as-prepared electrode in pH 7.4 HEPES buffer based on

differential pulse voltammetry (DPV). As shown in Fig. 1A, DPV peak currents increased with the increasing Ag^+ in the sample, indicating that the negatively charged ITO electrode could capture the Ag^+ in the solution through the electrostatic reaction as a result of the increasing peak current. Moreover, the modified electrode presented good electrochemical responses for the detection of Ag^+ at a concentration as low as 0.5 pM. Such a high sensitivity could provide a favorable facilitation for the following development of DNA sensor. As control test, we also investigated other positively charged ions, *e.g.*, Zn^{2+}, Cd^{2+} and Pb^{2+}. Results revealed that the negatively charged ITO electrode could exhibit certain DPV response. However, the peak potentials toward these cationic ions were not different each other (data not show). Thus, we could utilize the characteristic peak potential of silver ion to evaluate the Ag^+ concentration.

Next, we investigated the electrochemical characteristics of the negatively charged ITO electrode in the absence and presence of target DNA, using Ag^+-assisted hairpin DNA as the recognition element. As seen from Fig. 1B, DPV peak current in the presence of target DNA (curve '*a*') was obviously higher than that in the absence of target DNA (curve '*b*'). Logically, a puzzling question arises as to whether the increasing peak current originated from the added target DNA. To clarify this concern, 20 pM target DNA was directly added to pH 7.4 HEPES buffer. As indicated from curve '*c*', no peak current was obtained. The results suggested that the increasing peak current stemmed from the interaction between Ag^+-assisted hairpin DNA and target DNA, thereby leading to the dissociation of the chelated Ag^+ from hairpin DNA (Further investigation could be also found in Fig. 3).

Meanwhile, another two issues to be produced was whether (i) the chelated Ag^+ in the hairpins could stably stand into the hairpin in HEPES buffer, and (ii) target DNA could rapidly react with hairpin DNA. To verify this point, the designed strategy was utilized for the detection of 0 and 20 pM HIV DNA. The DPV peak current was collected and registered intermittently (every 30 min) (Fig. 2A). As seen from curve '*a*', the current was almost unchanged over 150 min in the absence of target DNA, indicating that silver ions could firmly intercalate into hairpin DNA. Upon target DNA introduction, however, the current increased with the increment of incubation time, and the steady-state signal was reached after 120 min (curve '*b*'). The results also suggested that (i) it took some time for hybridization reaction between hairpin DNA and target, (ii) target DNA could induce the dissociation of silver ion from hairpin DNA, and (iii) Ag^+-assisted hairpin DNA was relatively stable. To avoid possible error resulting from different additions of samples, all electronic signals obtained in this work were recorded at 120 min after addition of the samples.

Certainly, the used Ag^+ concentration during the preparation of hairpin DNA directly affected the sensitivity of DNA sensor. Too high-concentration Ag^+ caused the high background signal, which was not conducive for detection of low-concentration target DNA. However, a low concentration of silver ion was not favorable for the formation of hairpin DNA, thus resulting in a high detection limit. As shown from Fig. 2B, a highest signal-to-background current was achieved at 1 : 2.5 toward 20 pM HIV DNA (used as an example). Although a high-concentration Ag^+ in the detection solution could cause the increment in the DPV peak current, the background current also increased, thereby resulting in the decrement in the signal change (relative to background signal) (*Note*: excessive Ag^+ was not removed in this work). Considering this point, a 1 : 2.5 molar ratio of P_1 and Ag^+ was selected for preparation of hairpin DNA.

Under optimal conditions, the sensitivity and dynamic range of the electrochemical DNA sensor was monitored toward target DNA with different concentrations on the negatively charged ITO electrode in pH 7.4 HEPES buffer by using Ag^+-assisted hairpin DNA as the recognition element. Obviously, DPV peak currents displayed a dependence upon the concentration of target DNA, and the current increased with the increasing target DNA (Fig. 3A). As indicated from Fig. 3B, the increases in the current were proportional to target DNA concentration ranging from 1.5 pM to 50 pM, and the detection limit (LOD) was found at 1.0 pM target DNA estimated at the signal-to-noise ratio of 3σ. The sensitivity was obviously lower than those of fluorescence encoding-based microfluidic platform (14 nM),[9] polysaccharide-functionalized silver nanoparticles,[10] quadratic recycling amplification-based colorimetric assay (2.5 pM),[11] and hybridization chain reaction-based fluorescent assay (0.5 nM).[12]

The reproducibility of the electrochemical DNA sensor was evaluated by repeatedly assaying the high-middle-low target standards, using identical batches of DNA hairpins and the modified ITO electrodes. Results indicated that the relative standard deviations (RSDs) of the intra-assay between 6 runs were 8.9, 7.3 and 8.1% for 2.0, 20 and 40 pM target DNA, respectively, whereas the RSDs of the inter-assay with various batches were 9.4, 8.7 and 8.9% towards the mentioned-above analytes. So, the precision and reproducibility of this method was acceptable.

To study single nucleotide polymorphism (SNP) detection capability of our design, we used various oligonucleotides as perfect-matched target, single-base mismatched target, and non-complementary target. As shown in Fig. 4A, appreciable DPV peak currents were detected for both the perfect-matched target and the single-base mismatched target but not for the non-complementary target. Favorably, the change in the peak current (*vs*. blank current) induced by perfect-matched target was 1.59-fold higher than that by the single-base mismatched target toward the same-concentration target (20 pM), indicating the suitability of this method for SNP detection.

Finally, the electrochemical DNA sensor was also employed to determine the real samples by spiking HIV DNA standards into blank human serum specimens. As seen from Fig. 4B, the detectable electronic signals in the serum matrix were slightly low in comparison with those in the pure buffer as shown in Fig. 3. Moreover, the peak obviously became wide. The reason might be attributed to the interference of the sample matrix. Inspiringly, the presence of HIV DNA with different concentrations in the serum sample could be still differentiated from the control test, indicating the feasibility of our design for electronic monitoring of target HIV DNA in real physiological media.

In conclusion, we report the proof-of-concept of a new and powerful homogenous electrochemical monitoring platform for sensitive detection of HIV DNA based on hybridization-induced dissociation of silver ion from the hairpin DNA. Compared with conventional electrochemical DNA sensors, the system is simple, low-cost and user-friendly without the need of sample separation

and multiple washing steps. Nevertheless, only one disadvantage of this methodology requires a relatively long time to execute the hybridization reaction for the dissociation of silver ion. To fulfil the potential application for POC testing, future work should focus on improving the hybridization time.

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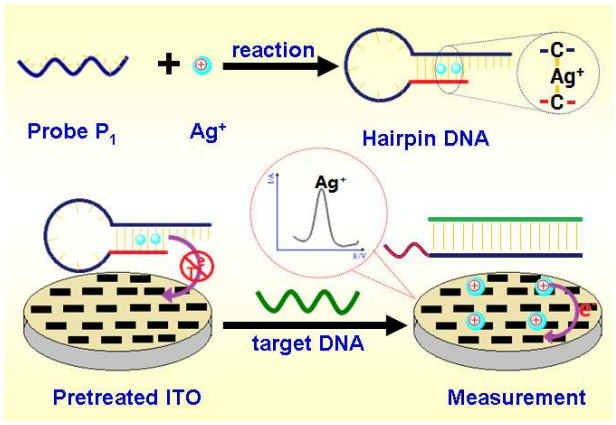

Scheme 1 Schematic illustration of hybridization-induced silver ion (Ag^+) dissociation from an immobilization-free and label-free hairpin DNA: (Top) Preparation of Ag^+-assisted hairpin DNA between probe P_1 and silver ion based on C-Ag^+-C coordination chemistry and (Bottom): measurement principle of the homogenous electrochemical sensor toward HIV DNA on a negatively charged ITO electrode.

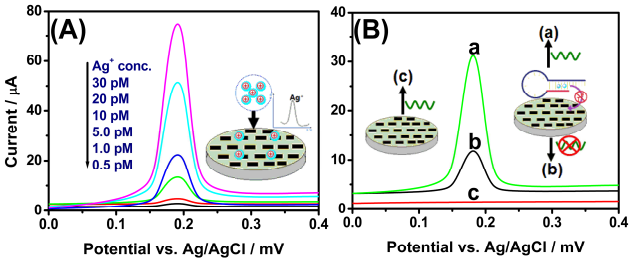

Fig. 1 (A) DPV responses of the negatively charged ITO electrode toward different-concentration silver ions in pH 7.4 HEPES buffer, and (B) DPV responses of the as-prepared hairpin DNA on the negatively charged ITO electrode in the (a) presence and (b) absence of 20 pM HIV DNA [*Note*: (c) the negatively charged ITO electrode toward 20 pM HIV DNA] in pH 7.4 HEPES buffer.

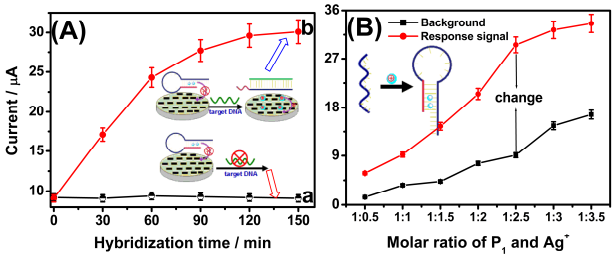

Fig. 2 (A) Electrochemical responses (versus hybridization time) of the hairpin DNA on the negatively charged ITO electrode in the (a) absence and (b) presence of target DNA (20 pM used in this case), and (B) the effects of molar ratio between probe P_1 and Ag^+ during the preparation of hairpin DNA on the electrochemical signal of the developed DNA sensor. Error bars indicate standard deviations (n = 3).

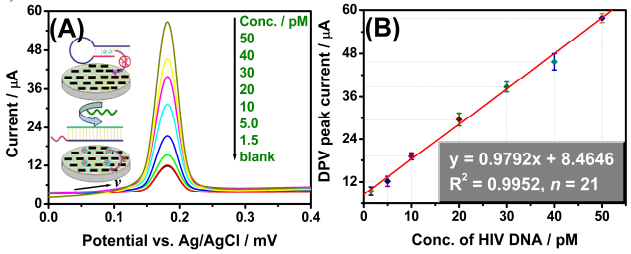

Fig. 3 (A) DPV responses of the homogeneous electrochemical DNA sensor toward HIV DNA with different concentrations in pH 7.4 HEPES buffer, and (B) the corresponding calibration curve (Parameters: Pulse amplitude: 50 mV; Pulse width: 50 ms; Potential: 0 – 400 mV). Error bars indicate standard deviations (n = 3).

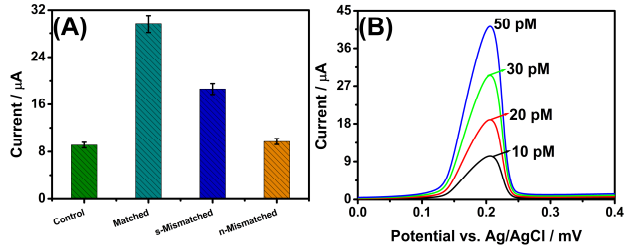

Fig. 4 (A) The specificity of the electrochemical DNA sensor against perfect-matched target (Matched), single-base mismatched target (s-Matched), and non-complementary target (n-Matched) (20 pM used in this case), and (B) DPV responses of the electrochemical DNA sensor toward the spiking HIV DNA in blank human serum samples with different concentrations.

Notes and references

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