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Signal-on CoA-dependent electrochemical biosensor for highly sensitive and label-free detection of Citrate synthase activity

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ABSTRACT

We report here a label-free and sensitive electrochemical method for probing Citrate synthase (CS) activity based on detailed investigations into the nucleic acid-mimicking coordination polymer (CP) formed from the coenzyme A (CoA)-Ag(I) repeat units. Our biosensing approach provides an especial and significant detection mechanism: CS can catalyze the essential condensation reaction between acetyl-coenzyme A (Ac-CoA) and oxaloacetate (OAA) to form citrate and CoA; then, in the presence of Ag(I), CoA-Ag(I) CP can be in situ formed because of the strong complexation ability of thiol groups of CoA toward Ag(I). The generated CoA-Ag(I) CP attaches to graphene-modified glassy carbon electrode surface by multiple adenine bases deriving from CoA and acting as the side groups along the polymeric backbone, which displays efficient H_2O_2-electrocatalyzing activity. More importantly, by using the formed polymer as signal output, the process is implemented to quantitatively analyze the activity of CS. Under the optimal conditions, CS with a detection limit as low as 0.00165 U/μL could be sensitively probed with a wide linear range from 0.0033 to 0.264 U/μL. Furthermore, with the character of label-free detection, high sensitivity and excellent selectivity, this strategy offers a convenient and specific method for CS activity detection and relevant inhibitors screening, which holds a promising potential in the practical application of CS-based biochemical research, disease diagnosis and drug discovery.

1. Introduction

The mitochondrial citric acid cycle (TCA cycle) plays an important role in central metabolic pathways of all aerobically living organisms [1,2]. Different carbon fuels are metabolized to acetyl-coenzyme A (Ac-CoA) and organic acids in aerobic cells, which are subsequently oxidized in the TCA cycle to deliver reduction equivalents for oxidation and ATP production in the mitochondrial electron transport chain. Citrate synthase (CS) catalyzes the first committed step of the TCA cycle, which is the aldol condensation of oxaloacetate (OAA) and acetyl-coenzyme A (Ac-CoA) to generate coenzyme A (CoA) and citrate [3]. As a rate-limiting enzyme of the cycle, CS is frequently regarded as a quantitative enzyme marker for the evidence of intact mitochondria [4]. Significantly, recent researches demonstrate that dysregulation of CS is closely associated with a variety of human diseases, especially metastatic cancers [5–7]. As a result, identification of CS activity and potential inhibitors is of utmost importance in the study of many fundamental metabolic processes and the development of both clinical diagnostics and cancer therapy.

So far, several approaches have been developed for the detection of CS activity and screening its inhibitors. Conventional approach is the UV–vis spectroscopic method, which is based on the reaction of CoA with the chromogenic thiol reagent 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) and therefore gives a changed absorption at 412 nm [8]. Then, various strategies have also been designed and successfully applied in CS activity detection, such as colorimetric assay [9], mass spectrometry [10], and product-indicated kit [11]. However, these CS-based assays suffer from some drawbacks, such as low detection sensitivity, laborious assay procedures, time-consuming and need of advanced equipment [12]. Therefore, some significant and viable alternatives, such as electrochemical method, should be considered as one of the most promising technologies for CS activity quantification because of its favorable portability, fast response, low cost and environmentally-friendly nature. Despite these merits, the related reported studies aimed at electrochemical detection of CS activity are quite scarce.

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Therefore, the development of a new, highly sensitive, selective, label-free and simple electrochemical method for the analysis of CS activity and screening for its potential inhibitors is highly desirable for the development and therapeutic application of CS-related medicine.

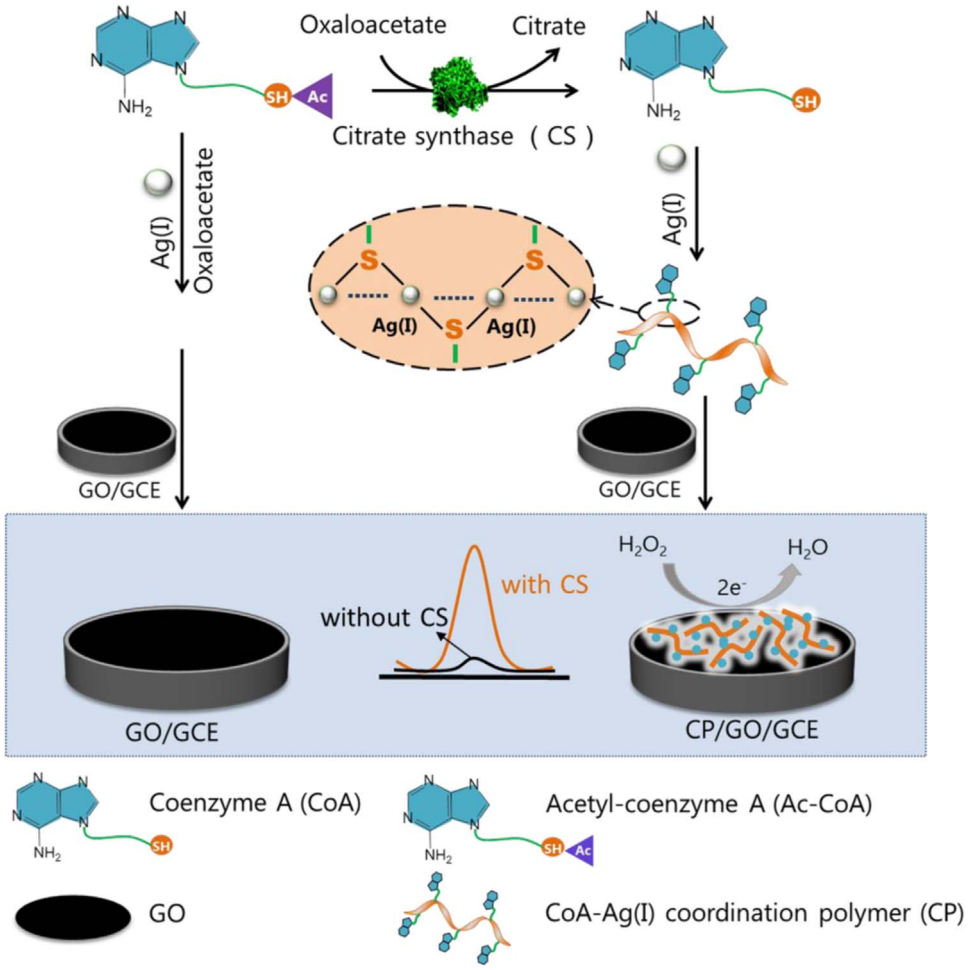
Recently, the thiol-Ag(I) coordination polymers (CP) as a type of novel materials have become extremely popular in various biological fields due to its unique luminescent, circular-dichroism and catalytic properties [13–16]. We recently showed that, in aqueous Ag(I) and coenzyme (CoA) solutions, CoA-Ag(I) CP can form in moderate environment, facilitated by thiol-Ag(I) interaction and Ag(I)· · ·Ag(I) interaction (termed argentophilic interactions) [15]. It is noteworthy that CoA, which is appealing as the ligand of CP ascribed to the free thiol and adenine at each end of its structure, is also a kind of product during CS-catalyzed reaction in the TCA cycle [15,17,18]. Currently, there is hardly any report about the utilization of CoA-Ag(I) CP for CS activity detection. Inspired by the proof-of-the-concept on CoA-Ag(I) CP, a much more convenient label-free electrochemical approach for the detection of CS activity with high sensitivity and excellent selectivity is presented there in this paper. The mechanism of CP-based CS activity sensing is explained by 1) the CS catalytic reaction, which involves the generation of CoA with unique structure; 2) high CP adsorbed on the graphene surface because of its structural similarity to poly-A nucleic acid; and 3) special signal output, which refers to electrocatalytic signal to H_2O_2 reduction. Scheme 1 shows the schematic diagram of the fabrication process of CoA-Ag(I) CP biosensor for the determination of CS activity. In the presence of CS, generated CoA leads to the formation of CP and produces apparent electrochemical signal. In this work, we not only discuss the mechanism and characteristics of the CP-based biosensor, but also present its analytical performance for determination of CS activity. This system is proposed to be used for conveniently and specifically monitoring the change of CS level in clinical samples and is also suitable for CS inhibitors screening.

2. Experimental section

2.1. Chemicals and instruments

Graphite powder (99.99995%, 325 mesh) was obtained from Alfa Aesar. The graphene oxide (GO) used in this work was prepared following Hummer's method [19]. Citrate synthase (CS) (suspension in 3.2 M $(NH_4)_2SO_4$ solution, pH 7.0), coenzyme A sodium salt hydrate (CoA), acetyl-coenzyme A (Ac-CoA), and oxaloacetate (OAA) were purchased from Sigma-Aldrich (Shanghai, China). All solutions were prepared with ultrapure water (18.25 MΩ cm), which was obtained from a Millipore Milli-Q system.

All electrochemical measurements were performed with a CHI 660 A electrochemical workstation (Chenhua, Shanghai, China) and a conventional three-electrode system was employed, which consisted of a glassy carbon working electrode, a platinum foil counter electrode, and a saturated calomel reference electrode (SCE). Fluorescence measurements were made using a QuantaMaster™ 4 fluorescence spectrometer (PTI). Fourier transform



Scheme 1. Schematic illustration of the electrochemical biosensor for probing Citrate synthase (CS) activity based on graphene oxide (GO) and electrocatalysis of nucleic acid-mimicking CoA-Ag(I) coordination polymer (CP).

infrared (FT-IR) spectra were obtained from a Nicolet AVATAR FT-IR360 spectrometer. UV–vis absorption spectra were performed on a Beckman DU-800 spectrophotometer. Dynamic light scattering (DLS) data were collected from a Zetasizer 3000 HS particle size analyzer (Malvern, UK). Atomic force microscope (AFM) images were performed on a Multimode 8 AFM (Bioscope system, Brucker) with tapping mode.

2.2. Fabrication of CP/GO/GCE

Prior to modification, glassy carbon electrode (GCE, 3 mm diameter) was sequentially polished on a microcloth with 1.0, 0.3 and 0.05 μm alumina suspension and successively sonicated with ultrapure water, ethanol, and ultrapure water for 5 min, and then dried in air at room temperature.

Then, the GO suspension (1.0 mg/mL) was prepared by ultrasonicating GO in HAc-NaAc buffer (0.1 M, pH 5.0) for 2 h. The graphene-modified glassy carbon electrodes (GO/GCE) were prepared by electrodepositing GO onto the surface of bare GCE and the cyclic voltammetry (CV) potential was cyclically swept from −1.5 to +0.5 V at 10 mV/s. Afterwards, 10 μL of the CoA-Ag(I) CP solution was dropped onto the surface of GO/GCE and the synthesis of CoA-Ag(I) coordination polymer (denoted as CoA-Ag(I) CP) is described in the Supporting Information. After incubation for 10 min, we rinsed the as-prepared electrode with phosphate buffer (10 mM, pH 7.0) to remove excessive CoA-Ag(I) CP. Finally, the prepared CP/GO/GCE was subjected to 50 mV/s cyclic voltammetry (CV) scans in phosphate buffer (0.1 M, pH 7.0, containing 5 mM H_2O_2) within the potential range from −1.2 to 0 V.

2.3. Electrochemical detection of Citrate synthase (CS) activity

For CS activity analysis, CS at various final concentrations (0, 0.00165, 0.0033, 0.0165, 0.033, 0.066, 0.132, 0.165, 0.198 and 0.264 U/μL) was incubated with Ac-CoA (200 μM), OAA (400 μM), and $AgNO_3$ (200 μM) in phosphate buffer (10 mM, pH 7.0) with a total volume of 100 μL at 25 °C for 1 h with gentle stirring. After that, the reaction solution was applied to further electrochemical measurements under the same conditions as those abovementioned.

2.4. Inhibition of CS studies

CS (0.264 U/μL) and ATP at various final concentrations (0, 0.5, 1, 3, 3.5, 5, 6, 6.5, 7, 8, 8.5, 9, 9.5 and 10 mM) were pre-incubated with Ac-CoA (200 μM) and OAA (400 μM) in phosphate buffer (10 mM, pH 7.0) with a total volume of 10 μL at 25 °C for 60 min. Then, certain amount of $AgNO_3$ (200 μM) and ultrapure water were added to the reaction solution (final sample volume 100 μL) and the mixture was incubated at 30 °C with gentle stirring (500 rpm) for 8 min. After that, the resulting mixture was used for further electrochemical measurement as described above.

3. Results and discussion

3.1. Principle of the assay

The principle of the label-free electrochemical strategy for the CS activity assay is illustrated in Scheme 1, which is based on a simple system of CoA-Ag(I) CP. CS, the first enzyme of the TCA cycle, is selected as the substrate and catalyzes the condensation reaction via transforming oxaloacetate (OAA) and acetyl-coenzyme A (Ac-CoA) into citrate and coenzyme A (CoA). CoA generated in TCA cycle reaction can be induced by Ag(I) to form a novel CoA-Ag(I) coordination polymer, which is facilitated by the thiol-Ag(I) interaction and aurophilic Ag(I)···Ag(I) interaction. Considering two kinds of special groups in CoA involving thiol and adenine, this proposed CP is given some special properties. One of them is the interesting -thiol-Ag(I)- repeated structure constructed in sequence via thiol-dependent formation with the inducement of Ag(I). Another interesting note is a considerable sum of adenines as side-chains along the polymeric backbone, resembling poly-A RNA. These unique features assure its high affinity with GO through non-covalent π-π stacking interaction. On the basis of this mechanism, an electrochemical assay is established by self-assembling CoA-Ag(I) CP onto a graphene-modified glassy carbon electrode (GO/GCE) surface and can electro-catalyze the reduction of H_2O_2, producing a strong electrochemical signal proportional to the amount of CoA. As a result, the highly sensitive CS activity assay can be realized by monitoring the change of the electrochemical signal. Therefore, the as-proposed label-free electrochemical strategy based on CoA-Ag(I) CP via thiol ligand in the presence of Ag(I) is a promising candidate for highly sensitive detection of the activity of CS and screening its inhibitor.

3.2. Assay of synthetic CoA-Ag(I) CP

It is well known that organothiolate anions (RS-) have strong complexation ability toward Ag(I) [20], so the formation of CoA-Ag(I) CP occurs immediately upon mixing CoA with Ag(I) in aqueous solution. This was indicated by a series of characterization experiments including UV–vis absorption spectra, DLS experiments, and FT-IR spectra. Fig. 1A shows an absorption band at around 330 nm after mixing CoA and Ag(I), which is assigned to the Ag(I)···Ag(I) interaction with ligand-to-metal charge transfer transition [21]. Dynamic light scattering (DLS) data also verifies the formation of CoA-Ag(I) CP in that the average hydrodynamic diameter corresponding to CoA-Ag(I) CP has significantly increased to *ca.* 164 ± 10 nm as the reaction between CoA and Ag(I) proceeds (Fig. 1B), coinciding with the previously reported thiol-Ag(I) CP [22]. In addition, FT-IR spectra (Fig. 1C) show that the characteristic stretching band of S-H at 2550 cm^{-1} can be clearly found in CoA that is absent in CoA-Ag(I) CP, further proving that S-H has been displaced by S-Ag interaction in the coordination polymer.

In addition, we also discovered that within this coordination polymer many adenines from CoA are included in the interaction network so that the polymeric chains are analogous to poly-A nucleic acid in terms of structure. To confirm this unique feature of CoA-Ag(I) CP, further fluorescence experiments were performed to investigate its ability to enhance the fluorescence of SYBR green II (SGII, a RNA-specific dye). As shown in Fig. 1D, it can be seen that the fluorescence intensity of CoA-Ag(I) CP stained by SGII (red) significantly increases compared to that of SGII-only control (black), sole CoA (green) or Ag(I) (blue). These comparative fluorescence data indicated that CoA promotes outstanding formation of the CoA-Ag(I) CP in the presence of Ag(I), which possesses a hallmark of poly-A RNA.

3.3. Affinity interaction between CoA-Ag(I) CP and GO

Given that structural similarity between CoA-Ag(I) CP and poly-A RNA, we therefore envisaged whether it could be a nucleic acid analogs structural platform to respond to GO via the strong π-π stacking interaction between adenine of CoA and carbon skeleton of GO. To test the affinity interaction between CoA-Ag(I) CP and GO, we carried out the fluorescence measurement to monitor the signal before and after centrifugation. As noted in Fig. 2A, the fluorescence intensity of CoA-Ag(I) CP stained by SGII at 530 nm is considerably higher than the background (curve a) regardless of undergoing the centrifugal treatment or not (curve c, b), indicating that almost all CoA-Ag(I) CP remains in the supernatant. In

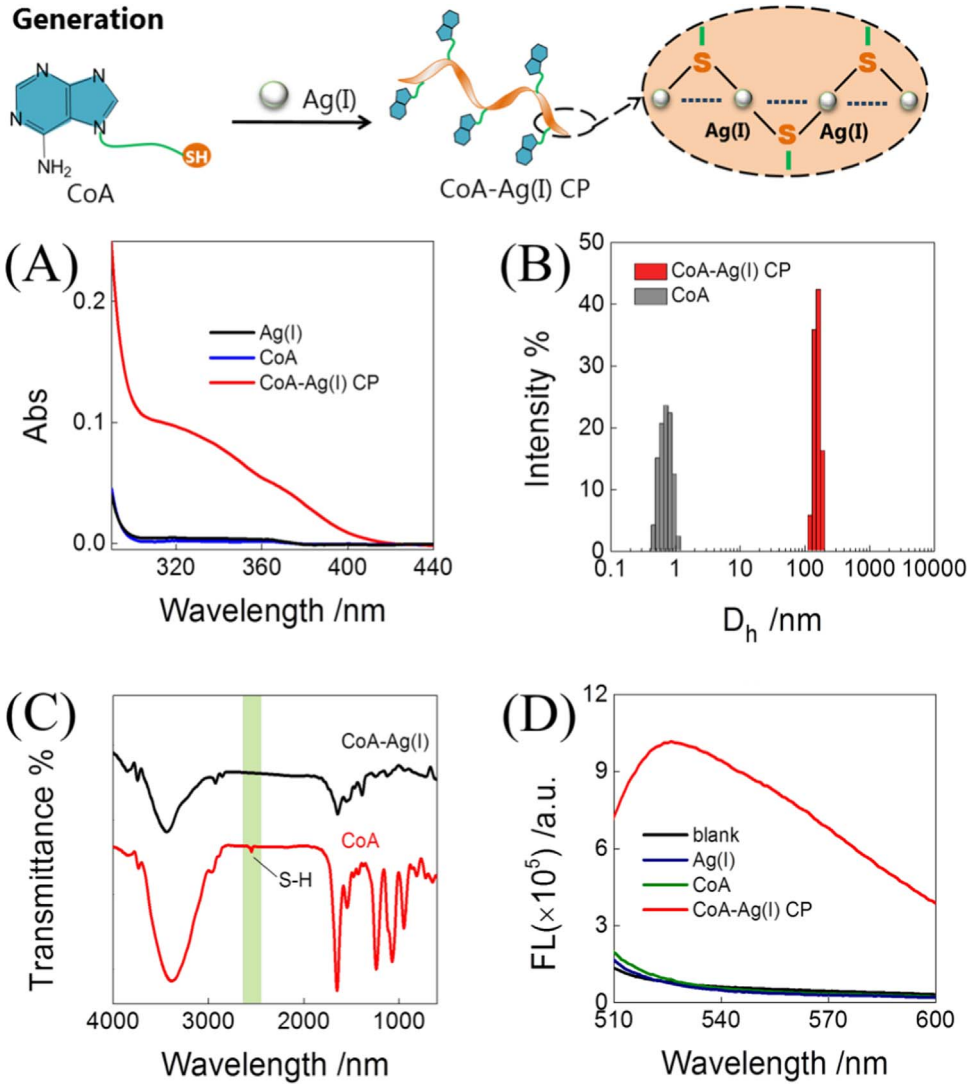


Fig. 1. (A) UV–vis absorption spectra of CoA-Ag(I) CP prepared by the reaction of Ag(I) and CoA in 0.1 M HAc-NaAc buffer at pH 5.0; (B) Hydrodynamic diameters (D_h) of CoA-Ag(I) CP determined by dynamic light scattering (DLS); (C) FT-IR spectra of CoA and CoA-Ag(I) CP; (D) Fluorescence spectra of SGII alone, Ag(I) with SGII, CoA with SGII, and the as-synthesized CoA-Ag(I) CP with SGII (λ_{ex}=494 nm). [CoA]=[Ag(I)]=100 μM. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)

contrast, introduction of GO to the CoA-Ag(I) CP solution and centrifugation of the mixture resulted in an obvious decrease in the fluorescence of the supernatant solution (curve d), suggesting that the CoA-Ag(I) CP precipitates together with GO.

Next, we further conduct AFM experiments to evaluate the combination of GO and CoA-Ag(I) CP (Fig. 2B, Fig. S1). In all cases, the CP/GO combinations yielded a number of wire-like substances with different lengths and sizes on GO surface (Fig. 2B, Fig. S1D). Importantly, the average height of the CoA-Ag(I) CP is around 2 nm, as observed from its associated height profiles (Fig. 2B inset), consistent with the diameter of double-stranded DNA (dsDNA) helix [23]. Moreover, large-area AFM images of CoA-Ag (I) CP and GO further illustrate the binding interaction between GO and CoA-Ag(I) CP (Fig. S1E, F). However, when GO is mixed with either CoA or Ag(I) (Fig. S1B, C), hardly any chain-like substance in AFM images can be found, which is the same as that of the sole GO (Fig. S1A). Thus, it is evident that the CoA-Ag(I) CP can interact with GO on the basis of all the above results.

Additionally, we also employed electrochemical measurements to further test the interaction between CoA-Ag(I) CP and GO. As shown in Fig. 2C, compared with bare GCE (black), the CV signal of $[Fe(CN)_6]^{3-/4-}$ at the GO/GCE (blue) dramatically increases, indicating effective electron transfer by GO modification. However, the CV signal of the GO/GCE with adsorbed of CoA-Ag(I) CP (CP/GO/GCE) (red) significantly decreases, which is likely to arise from the electrostatic repulsion between phosphate groups in CoA-Ag (I) CP and anionic $[Fe(CN)_6]^{3-/4-}$. Fig. 2D shows the electron transfer resistance (R_{et}) of above-mentioned modified electrodes using electrochemical impedance spectra (EIS). EIS of the bare GCE (black) appears as a small semicircle and subsequent modification of GO (GO/GCE) (blue) exhibits a definitely lower semicircle due to the well conductivity of GO. Nevertheless, at the CP/GO/GCE (red), the R_{et} has obviously increased, while no change of R_{et} on CP/GCE (purple) can be found compared with that of the bare GCE. Combining all the above observations achieved in fluorescent spectra, AFM experiments, CV and EIS studies, we confirm that CoA-Ag (I) CP can be adsorbed firmly onto the GO surface.

3.4. Electro-catalytic reduction of H_2O_2 by CP/GO/GCE and detection of CoA

Recently, our group and other groups have demonstrated that the DNA-templated silver nanoclusters (DNA-AgNCs) loaded on GO/GCE are able to catalyze the electrochemical reduction of H_2O_2

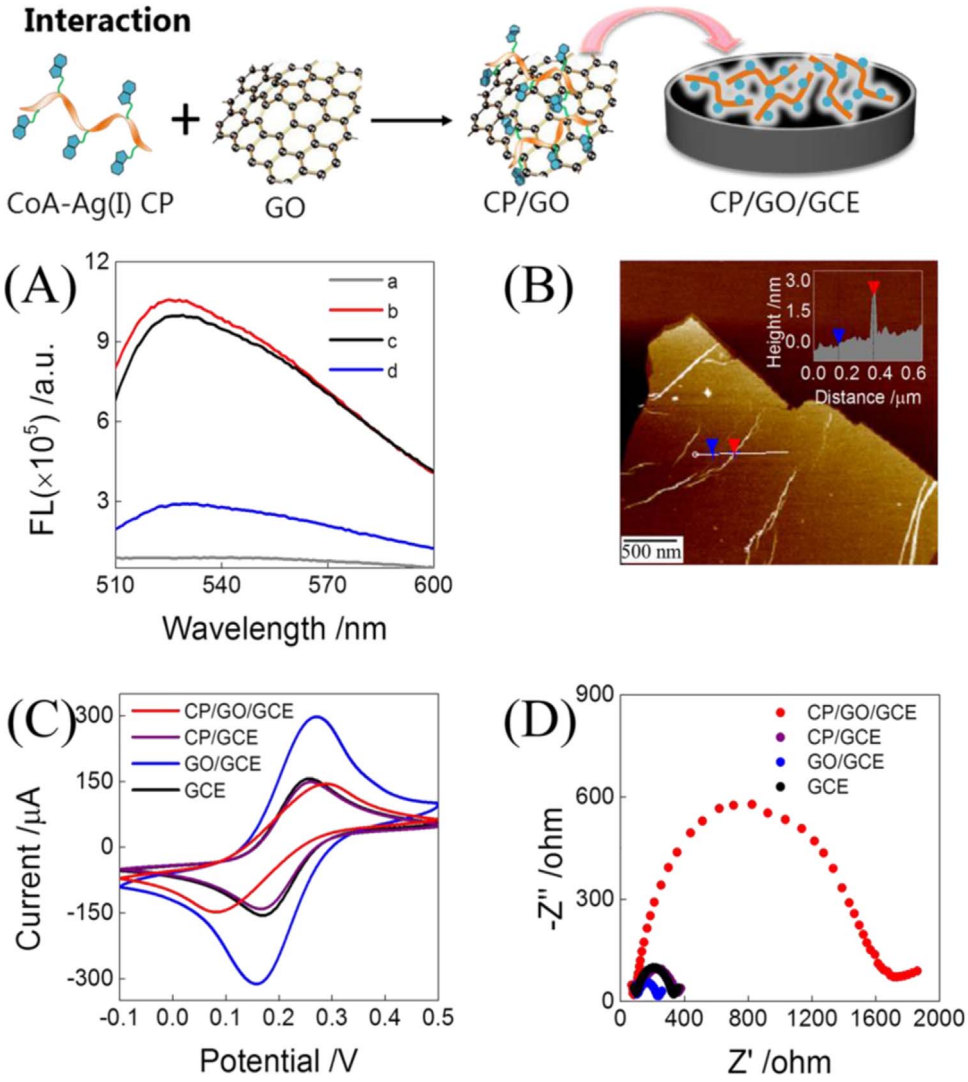


Fig. 2. (A) Fluorescence experiments for proving the binding interaction of GO and CoA-Ag(I) CP in the presence of SGII: (a) SGII alone, (b) CoA-Ag(I) CP, (c) the supernatant of CoA-Ag(I) CP after centrifugation, and (d) the supernatant of mixture of CoA-Ag(I) CP and GO after centrifugation; (B) Atomic force microscopy (AFM) image of the mixture of CoA-Ag(I) CP and GO. Inset: the associated height profile of CoA-Ag(I) CP; (C) Cyclic voltammetry (CV) responses and (D) electrochemical impedance spectra (EIS) of different modified electrodes: GCE (black); GO/GCE (blue); CP/GCE (purple); CP/GO/GCE (red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

[24,25]. Since CoA-Ag(I) CP is structurally analogous to nucleic acid and also silver-containing, it is reasonable that CoA-Ag(I) CP may electro-catalyze H_2O_2 reduction. Fig. 3A, B, C show the typical CVs for H_2O_2 reduction by various modified electrodes during the fabrication process in the PBS buffer (0.1 M, pH 7.0) containing 5 mM H_2O_2. An outstanding CV peak at −0.7 V corresponding to H_2O_2 was observed for CP/GO/GCE surface compared to that of CoA/GO/GCE, Ag(I)/GO/GCE, CP/GCE or GO/GCE. Furthermore, there is no apparent peak current in the absence of H_2O_2 (Fig. 3D), further proving that the current is generated via electro-catalytic activity of CP/GO/GCE toward H_2O_2. The reason for CoA-Ag(I) CP-induced electro-catalytic reduction may arise from that the Ag (I) in CoA-Ag(I) CP is simultaneously reduced to CoA-Ag(0) CP or tiny AgNCs in the electrochemical scanning process and the resulting CoA-Ag(0) CP or tiny AgNCs, which can enhance electrocatalysis towards H_2O_2, are attributed to the electro-catalytic reduction [15,26,27]. Previously, we have showed that dominant silver species in original CoA-Ag(I) CP is Ag(I), which is subsequently reduced to Ag(0) of the coordination polymer during H_2O_2-electrocatalyzing process [15]. In order to further study whether the reduced Ag(0) accounts for the electrocatalytic reduction of H_2O_2, we directly electrodeposited Ag(0) on the GO/GCE (Ag(0)/GO/GCE) and the results are illustrated in Fig. S2A. A high reduction peak current was observed for the Ag(0)/GO/GCE in the presence of 5 mM H_2O_2 compared with the GO/GCE and the reduction peak was appeared at −0.55 V. However, no obvious reduction peak was observed for the Ag(0)/GO/GCE without H_2O_2, further indicating the electrocatalytic activity toward H_2O_2 of Ag (0)/GO/GCE. After that, according to the literature [15], we also prepared CoA-capped silver nanoclusters (CoA-AgNCs) introducing reductant $NaBH_4$ to CoA-Ag(I) CP solution as a control to convince the Ag(0) mechanism and found that AgNCs-loaded GCE (AgNCs/GO/GCE) also can efficiently electrocatalyze the reduction of H_2O_2 (Fig. S2B). Taken together, the plausible mechanism could be deduced as follows:

$$\text{CoA-Ag(I) CP} + e^- \rightarrow \text{CoA-Ag(0) CP at the electrode} \quad (1)$$

$$2\text{CoA-Ag(0) CP} + H_2O_2 \rightarrow 2\text{CoA-[Ag(I)-OH] CP} \quad (2)$$

$$2\text{CoA-[Ag(I)-OH] CP} + 2e^- + 2H^+ \rightarrow 2\text{CoA-Ag(0) CP} + H_2O \quad (3)$$

Based on the above-mentioned results, we turned our attention

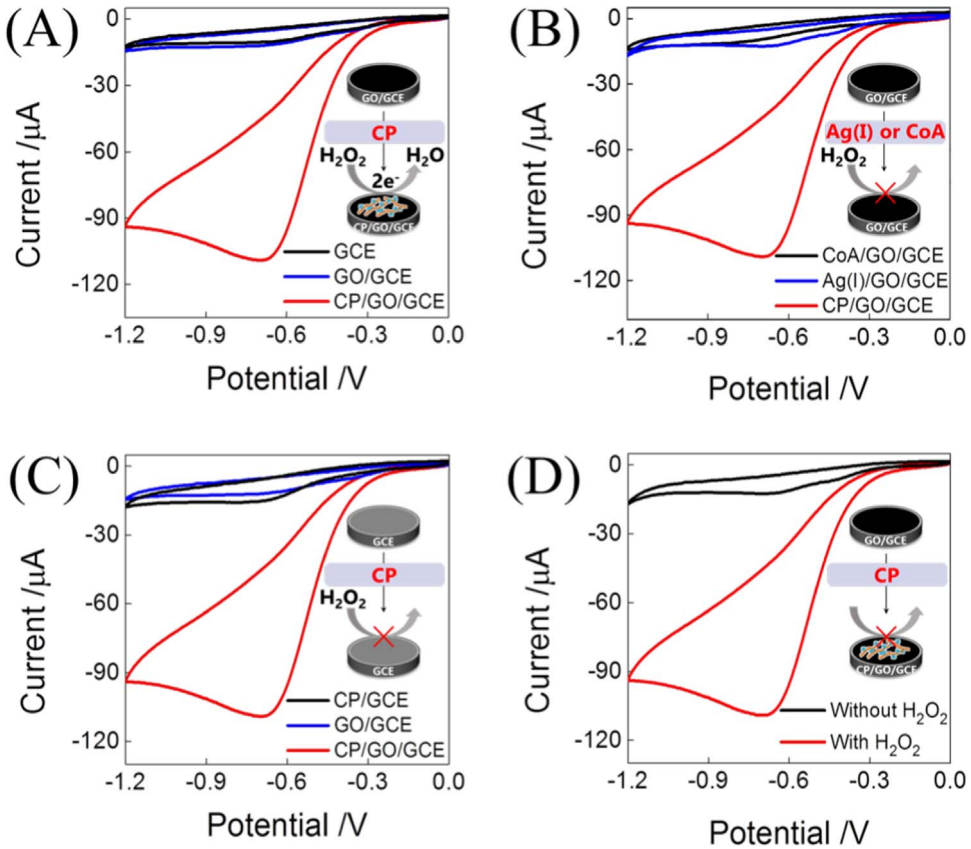


Fig. 3. Cyclic voltammetry (CV) responses of different modified electrodes in N_2-saturated PBS (0.1 M, pH 7.0, containing 5 mM H_2O_2): (A) bare GCE (black), GO/GCE (blue) and CP/GO/GCE (red); (B) CoA/GO/GCE (black), Ag(I)/GO/GCE (blue) and CP/GO/GCE (red); (C) CP/GCE (black), GO/GCE (blue), and CP/GO/GCE (red); (D) Cyclic voltammetry (CV) of CP/GO/GCE in responses to absence (black) and presence (red) of 5 mM H_2O_2 in N_2-saturated PBS (0.1 M, pH 7.0). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

to the detection of CoA, since detecting this unique small biomolecule has been an active research goal. Under the optimal conditions, we assessed the CV response of the sensor relative to the concentration of CoA. As shown in Fig. S3A, the electro-catalytic current linearly increases along with the concentration of CoA increasing from 0 to 100 μM, indicating the increasing amount of CoA-Ag(I) CP loaded on the GO/GCE. A linear relationship between the electro-catalytic current and the CoA concentration is shown in Fig. S3B, and the detection limit is 0.02 μM (S/N=3). The proposed sensor presents an efficient mechanism for electrochemical detection of CoA. To evaluate the practicality of the proposed method, a recovery experiment by analyzing CoA in human serum samples was carried out. Five levels of 10, 20, 40, 60, and 100 μM CoA were spiked to human serum samples respectively, using standard addition technique. The test results are listed in Table S1, and satisfactory recoveries are obtained, namely, 94.6–105.2%.

3.5. Probing Citrate synthase (CS) activity

As is well known to us, Citrate synthase (CS) has been shown to play significant role in regulating TCA cycle which can catalyze the synthesis of CoA and citrate from OAA and Ac-CoA. It is therefore understandable that the proposed method can be applied to the assay of CS activity. Compared to conventional methods for detection of CS activity, our electrochemical assay is more sensitive, specific, cost-effective and time-saving, as shown in Table S2. The principle of the electrochemical method for assaying CS activity is depicted in Scheme 1. The reactant Ac-CoA contains an acetyl group linked with its sulfhydryl portion, which hampers the generation of the coordination polymer. If the reaction happens, it should generate CoA as a product in the presence of CS, which can interact with Ag(I) to form CoA-Ag(I) CP. CoA-Ag(I) CP readily loaded on GO/GCE (CP/GO/GCE) has the ability to efficiently electro-catalyze H_2O_2 reduction, so that CS activity can be facilely probed by monitoring the generation of CoA. The current intensity is directly dependent on the amount of CS added to the reaction solution. Hence, the activity of CS can be quantitatively measured by monitoring CoA generation during the enzyme-catalyzed reaction. As shown in Fig. 4A, we tested the feasibility of the proposed sensor for CS activity detection. The results show that the CS reaction mixture leads to noticeable current response. However, in the absence of any of these reactants, it only presents a weak signal, which can be neglected. The quantitative detection of CS activity was performed with different concentrations of CS (Fig. 4C). An obvious CV peak current of H_2O_2 at −0.7 V is observed and the electrochemical response current of CP/GO/GCE increases gradually with the increment of concentration of CS. The calibration curve of the CV responses at different CS concentrations is shown in Fig. 4D, the linear range is from 0.0033 to 0.264 U/μL and the obtained regression coefficient R is 0.9823. The limit of detection is down to 0.00165 U/μL.

3.6. Selectivity and storage stability of the proposed sensor

The high selectivity of the assay is crucial for a biosensor to analyze the complex biological samples. Since we have demonstrated that our proposed sensor exhibits high sensitivity to different concentrations of CoA and the specificity of the proposed sensor to CoA was also elucidated by challenging it with some ubiquitous thiol-containing compounds and adenine-containing

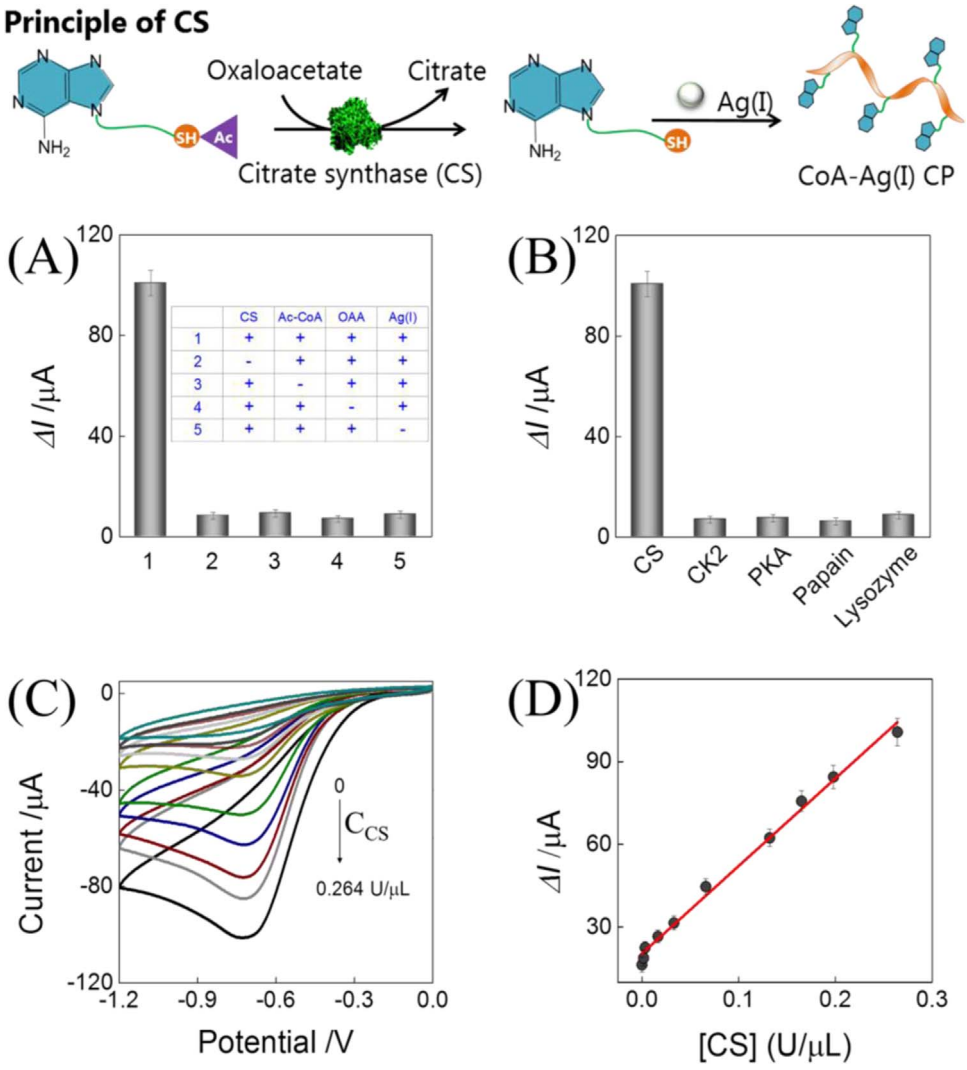


Fig. 4. (A) Electrochemical detection of CS activity by the proposed sensor. [CS]=0.264 U/μL, [Ac-CoA]=200 μM, [OAA]=400 μM, and [Ag(I)]=200 μM; (B) Selectivity of the proposed sensor for assay of 0.264 U/μL of CS, CK2, PKA, Papain, and Lysozyme; (C) Cyclic voltammetry (CV) responses of the proposed sensor towards different concentrations of CS (from top to bottom: 0, 0.00165, 0.0033, 0.0165, 0.033, 0.066, 0.132, 0.165, 0.198, and 0.264 U/μL) in N_2-saturated PBS (0.1 M, pH 7.0 containing 5 mM H_2O_2); (D) The linear relationship between the peak current and CS concentration based on the proposed electrochemical CS sensor.

compounds. They were glutathione (GSH), *L*-cysteine (*L*-Cys), tripeptide (GCG), adenosine triphosphate (ATP) and Ac-CoA. As we can see from Fig. S4, none of these compounds gave any noticeable electrochemical signal, revealing that both the thiol group and the adenosine are essential for the proposed platform. Therefore, the proposed sensor shows remarkably high selectivity to CoA.

Furthermore, considering the important role of CS in regulation of TCA cycle, the selectivity of the CS sensor was evaluated by using other common proteins: Protein kinase (PKA), Creatine kinase (CK2), Lysozyme and Papain. As shown in Fig. 4B, under the same experimental conditions, the electrochemical signal for CS is much higher than that of other enzymes, implying an excellent selectivity. In addition, the storage ability of the proposed sensor was also checked. It is found that the response of the sensor to 5 mM H_2O_2 has no obvious change in the continuous 7 days by using it every two days (Fig. S5). After a month, the electrochemical current relative to original current in the 1-st measurement is approximately 88%, suggesting a good and long-term stability of the sensor. Such results indicate that the CoA-Ag(I) CP-based electrochemical sensor is a potent platform for the sensitive detection of CS activity.

3.7. Inhibitor screening

It is of great importance to screen and analyze the inhibitors of CS in the therapeutic application of CS-related drug. Therefore, we also applied our method for assaying the potential CS inhibitor, ATP, to test the feasibility of the sensor in inhibitors screening. Briefly, a series of different concentrations of ATP was premixed with the CS reaction mixture before introducing Ag(I) and the inhibitor would compete with Ac-CoA to lead to a decrease in the affinity of the CS for Ac-CoA. After mixing with Ag(I), the mixture was dropped on the GO/GCE to collect electrochemical signal, as illustrated in Fig. 5A. We found that the CS activity is apparently inhibited with the addition of ATP and the corresponding electrochemical signal is decreased accordingly dependent on the amount of ATP added. Meanwhile, it can be seen that the electrochemical current intensity decreases about 84.4% with the addition of 10 mM ATP and the relationship between response current and ATP concentration is plotted in Fig. 5B, from which the IC_{50} value (half maximal inhibitory concentration) of 0.264 U/μL is calculated to be 5.48 ± 0.06 mM. These observations clearly suggest that the CoA-Ag(I) CP-based sensor can be used for the quantitatively screening of CS inhibitors.

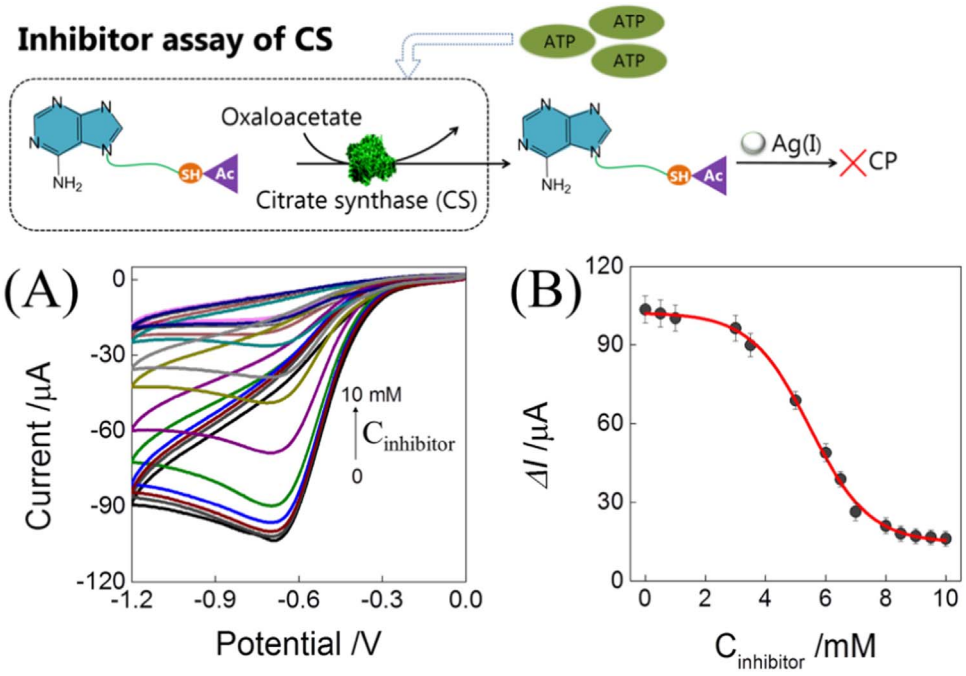


Fig. 5. (A) Cyclic voltammetry (CV) of the proposed biosensor in response to different concentrations of CS inhibitor (ATP) (ranging from 0 to 10 mM); (B) Relationship between electrochemical current intensity and the inhibitor concentration. [CS]=0.264 U/μL.

4. Conclusion

In summary, the present study proposed a highly sensitive and selective electrochemical method for CS activity detection and inhibitor screening. It is worth noting that CoA, the product of CS catalysis, possesses a unique structural characteristic containing both the thiol group and adenosine base. Optimization of the experimental conditions could yield self-assembled nucleic acid-mimicking coordination polymer with the addition of Ag(I) to CS-related reaction system. The formed CoA-Ag(I) CP with similar nucleic acid structure could attach to GO surface, which provides a new sight into the fabrication of electrochemical sensor. We found two critical factors for the strategy: thiol and adenine group. Thiol is required to participate in the assembly of CoA-Ag(I) CP, and adenine is a prerequisite to the binding of CoA-Ag(I) CP and GO. The electrochemical measurements showed that the obtained CoA-Ag(I) CP exhibits high electro-catalytic activity for H_2O_2 reduction and they can be used as electrochemical sensing materials for sensitive and selective detection of CS activity with a detection limit as low as 0.00165 U/μL. What's more, this assay could be further employed for monitoring the variation of the CS activity in response to a CS-relevant inhibitor. Continued development of this facile and label-free detection method will allow for a better understanding of the basic role of CS in the TCA cycle and contribute to disease diagnosis, therapy and CS-related drug screening.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2016.08.085.

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