Amperometric indicator displacement assay for biomarker monitoring: indirectly sensing strategy for electrochemically inactive sarcosine

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Abstract

Indicator displacement assay plays a fundamental role in the development of chemosensors. We explored here an ingenious yet effective strategy for amperometric assay of electrochemically inactive sarcosine based on an indicator displacement principle, in which 1,2-naphthoquinone-4-sulphonic acid sodium salt (NQS) was proposed as the receptor and the electroactive $\text{Ru}(\text{NH}_3)_6^{3+}$ cations used as an indicator. Due to the stronger binding affinity of the NQS toward sarcosine than toward $\text{Ru}(\text{NH}_3)_6^{3+}$, the developed amperometric indicator displacement assay (A-IDA) exhibits high selectivity and excellent sensitivity toward sarcosine determination as well as with a lower detection limit (30.00 nM, S/N = 3).

Keywords: Indicator displacement assay; Amperometric sensor; Sarcosine; Colorimetric sensor; Electrochemical inactive target

1. Introduction

With the rapid development of indicator displacement assay (IDA) principle, its combination with different analytical technology actively promotes significant progress in analytical chemistry [1]. As well known, IDA traditionally signals the binding of the analyte based on the molecular design of receptor and indicator that contains a “binding site” and “signaling site” [2, 3]. Once the analyte associated with the binding site, a microenvironment modulation would occur thus further perturbs the properties of the signaling site and results in producing an obvious change with sensitive signal readout such as optical or electrochemical responses. To date, many unique indicators with built-in “signaling sites” and synthetic receptors containing potential “binding sites” have been extensively used to construct IDA based colorimetric sensors for different objectives including anions [4], cations [5] and biomolecules [6, 7] and so on [8]. Due to a sensitive modulated signal, those IDA based colorimetric sensors have explored many advantages such as

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sensitive color change, easy and convenient to control, and works well in aqueous and organic media.

Although IDA principle plays a key role in sensing techniques, it has seen relatively mandatory requirement on the geometry of the receptor and/or the indicator, its charge, its hydrophobicity, and also the solvent system redesign, which may block the achievement of the simple and facile colorimetric sensor. In addition, for a highly selective and sensitive IDA sensor, another important factor of analytical technique to detect the affinity between the receptor and indicator or the analyte, typically leading the monitoring process into a series of disadvantages, such as complicated operation, expensive equipment, time consuming and others [9, 10]. To overcome these, many efforts have been attempted on the strategies through covalently associating the colorimetric or fluorescent indicator to a unique receptor, followed by detecting the occurred optical change with optical instruments such as UV-vis and fluorescence spectrum technology [11, 12].

As is well known, electrochemical methods have more benefits in various analytical purposes due to their high sensitivity, wide linear range, and low-cost instrumentation [13-18]. However, the use of IDA principle on the fabrication of novel electroanalytical sensor have scarcely been explored so far [19]. On the other hand, the direct amperometric assay of electrochemical inactive or inherent objectives as a longstanding challenge in electroanalytical field because of poor electrochemical properties of those analytes [20-24]. For example, sarcosine, an important biomarker of prostate cancer (PCa), sensitive determination of sarcosine levels is more useful for the construction of non-invasive diagnostic methods of PCa. But the direct electroanalysis of sarcosine appears to be appealing alternatives due to its intrinsic disadvantages on the information deriving and gathering [25].

Based on this, we set out to develop a facile yet effective electrochemical method for sarcosine determination by combining IDA principle with amperometric technology (A-IDA). Compared to other electrochemical sensors of sarcosine, the proposed electrochemical platform has many advantages. For example, the construction of the proposed modified electrode without any loss of enzyme immobilization and complicated synthesis, which may not use complicated operation and much time. Furthermore the sensing strategy of electrochemically inactive sarcosine first combined IDA principle with electrochemical assay and the sarcosine target was easily

detected by the decrease of the electrochemical signals. More importantly, our A-IDA principle opens a novel route to sense an electrochemically inactive sarcosine through an indirectly electrochemical strategy.

2. Material and methods

2.1. Materials and preparation of A-IDA

Graphene oxide (GO) was purchased from Nanjing XFNANO Materials Tech Co., Ltd., (Nanjing, China). 1,2-naphthoquinone-4-sulphonic acid sodium salt (NQS), Hexaammineruthenium ($\text{Ru}(\text{NH}_3)_6^{3+}$), sarcosine and other chemicals were provided by Aladdin. The proposed A-IDA was fabricated according to the procedures as follows. First, glassy carbon electrode (GCE, 3 mm) was carefully polished with Al_2O_3 slurry before the coating of resultant homogeneous GO solution (3 μL). Then the as-gained GO modified GCE was immersed into 0.1 M KCl solution containing 5 mM NQS and suffered cyclic voltammetry scan for 60 cycles within the potential range of -0.50 V to -0.10 V to achieve the NQS/GO modified GCE. Subsequently, the NQS/GO modified GCE was immersed in 0.10 M KCl containing 5 mM $\text{Ru}(\text{NH}_3)_6^{3+}$ and suffered cyclic voltammetry scan for 40 cycles within the potential range of 0 V to -0.50 V and achieved the $\text{Ru}(\text{NH}_3)_6^{3+}$ /NQS/GO modified GCE. $\text{Ru}(\text{NH}_3)_6^{3+}$ /GO and $\text{Ru}(\text{NH}_3)_6^{3+}$ /NQS modified GCE were prepared at the same condition.

2.2. Characterization and apparatus

Cyclic voltammograms and differential pulse voltammograms were acquired on a CHI660 electrochemical station (CHI Instruments Inc., USA) with a conventional three-electrode system using bare and above modified GCE as working electrode, platinum wire and Ag/AgCl (saturated-KCl) as auxiliary and reference electrode, respectively. Scanning electron microscopy (SEM, Zeiss, Oberkochen, Germany) equipped with an energy-dispersive X-ray spectrometer (EDS) (Aztec-X-80, Oxford) operating at an accelerating voltage of 5 kV.

2.3. Amperometric IDA measurements

Before electrochemical measurements of sarcosine, the $\text{Ru}(\text{NH}_3)_6^{3+}$ /NQS/GO modified GCE was dipped into 0.10 M KCl aqueous solution for 40 min until stable redox peaks obtained (Fig. S1). Then it was immersed into sarcosine solution containing 0.10 M NaOH- H_3BO_3 buffer solution (pH, 10.00) for 10 minutes and taken out the electrode and successively rinsed with

doubly distilled water. After that, it was immersed into 0.1 M KCl solution to achieve electrochemical measurement.

3. Results and Discussion

The successfully fabricate an A-IDA for the determination of electrochemically inactive sarcosine, a natural NQS and $\text{Ru}(\text{NH}_3)_6^{3+}$ was proposed as the receptor and an indicator, respectively. As elucidated in Fig. 1A, NQS molecules with unique recognition sites were directly modified on a GO modified GCE surface through electropolymerization (Fig. S2) by forming a sensitive composite substrates with GO, on which $\text{Ru}(\text{NH}_3)_6^{3+}$ species were further successfully modified and stably confined due to the goodly electrostatic binding between $\text{Ru}(\text{NH}_3)_6^{3+}$ and sulfonic acid groups of NQS molecules (Fig. S3) [26]. Such a modified electrode exhibits a good voltammetric response with a higher current in 0.10 M KCl due to the stably confined $\text{Ru}(\text{NH}_3)_6^{3+}$ species on the electrode surface. Interestingly, NQS receptor exhibits a stronger affinity toward sarcosine than toward the indicator of $\text{Ru}(\text{NH}_3)_6^{3+}$, and therefore result in a typical indicator displacement of $\text{Ru}(\text{NH}_3)_6^{3+}$ species on the electrode surface due to the unique nucleophilic substitution reaction between the amine group of sarcosine and NQS receptor (Fig. 1B and Fig. S5) [28, 29]. As a result, the redox behaviours of $\text{Ru}(\text{NH}_3)_6^{3+}$ species on the $\text{Ru}(\text{NH}_3)_6^{3+}$ /NQS/GO modified GCE surface would be changed as well as with a lower current response, which can quantitatively reflect the amount of analytes (Fig. S6).

To explore the effect of GO film, the comparative experiments were performed (Fig. S4). These results indicated that the GO film not only increases the amount of NQS molecules through π - π stacking interaction between GO with NQS molecules, but also enhances the amount of $\text{Ru}(\text{NH}_3)_6^{3+}$ species due to the electrostatic binding between sulfuric acid group of NQS molecule and $\text{Ru}(\text{NH}_3)_6^{3+}$ specie, which will be beneficial for the analytical performance of the proposed electrochemical sensor toward sarcosine analysis. Furthermore the surface adsorption of $\text{Ru}(\text{NH}_3)_6^{3+}$ and sarcosine on the negative charge surface of GO have significant contributions to the electrochemical response signal of our developed sensor. As shown in Fig. S3 and Fig. S4, this result revealed that the effect of GO film is beneficial for increasing the electrochemical response signal of $\text{Ru}(\text{NH}_3)_6^{3+}$ and enhancing the amount of the sarcosine for nucleophilic substitution reaction between the sarcosine and NQS receptor. However, due to the electrical double layer

screening of GO sheets, increasing ionic concentration such as K^+ , Cl^- and others will suppress the electrostatic repulsion between the negatively charged GO sheets and other molecules. Furthermore, the electrolyte ions can also affect the adsorption by ion exchange competition with adsorbate ions, therefore the decrease in electrostatic interactions between NQS molecules and $Ru(NH_3)_6^{3+}$ with the increase in ionic strengths [27]. To avoid these difficulties, the concentration of electrolyte and buffer solution has been typically chosen as 0.10 M.

To address above mechanisms, proof-concept-experiments were performed by using UV-vis spectroscopy and electrochemical methods. Initially, the different binding affinity of NQS receptor toward $Ru(NH_3)_6^{3+}$ and sarcosine was confirmed by using UV-vis spectroscopy (Fig. 2A) [26, 30]. The obtained differences on the absorption peak as well as an obvious color change strongly demonstrate the fact that the proposed receptor exhibits stronger binding affinity toward the analyte than toward the indicator. Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) with higher S/N were also used to evaluate the possibility of the proposed A-IDA for electrochemically quantitative analysis toward sarcosine. Firstly, typical redox peaks of the $Ru(NH_3)_6^{3+}$ /NQS/GO modified GCE in 0.10 M KCl within a given potential range from 0 to -0.5 V were well displayed (black curve in Fig. 2B, and Fig. S7) with CV and DPV (black curve in Fig. 2C, and Fig. S8), which can be attributed to the electron transfer process of electroactive $Ru(NH_3)_6^{3+}$ species that stably confined on the modified electrode surface. In addition, to insure a good stability, the $Ru(NH_3)_6^{3+}$ /NQS/GO modified GCE was immersed into 0.10 M KCl solution for 40 min until a stable voltammetry response was gained (Fig. S1). Subsequently, the electrode was immersed in a sample solution that containing 48.70 μ M sarcosine for 10 min so as to achieve an indicator displacement between sarcosine and $Ru(NH_3)_6^{3+}$ on the electrode surface (Fig. S9). After taken out from the sample solution, the electrode was repeatedly rinsed with water and measured by using CV and DPV methods in 0.10 M KCl solution. As depicted (red curve in Fig. 2B and Fig. 2C), the current of the $Ru(NH_3)_6^{3+}$ /NQS/GO modified GCE was obviously decreased compared to that of without suffering the treatment of sarcosine. However, no obvious change in the current response was observed after the $Ru(NH_3)_6^{3+}$ /GO modified GCE was immersed in 48.70 μ M sarcosine for 10 min, as typically depicted in Fig. S10, strongly indicating that the proposed mechanism could be verified. The proposed sensing process was also checked by using scanning electron microscopy (SEM) and energy-dispersive X-ray spectrometer (EDS), respectively. As

well displayed (Fig. 3-Fig. 4), obvious change on the nanoscale microstructures of different electrodes and a detectable amount of C, N, O, S and Ru at 0.26, 0.39, 0.51, 2.38 and 2.68 keV strongly indicating that the electroactive $\text{Ru}(\text{NH}_3)_6^{3+}$ species on the electrode surface suffered dissociation due to the addition of sarcosine and therefore provided an electrochemical signal modulation.

The sensing performance of our A-IDA to the target was verified by using DPV technology. As well displayed (Fig. 5A), the response current of the proposed A-IDA gradually decreases with increasing the concentration of sarcosine, demonstrating the developed assay possesses a sensitive electrochemical readout signal for the target. To eliminate the variation of electrode-to-electrode in the background signal, the ratio of current decrease (R) for sarcosine quantification was proposed in this work [19]. Here, R was defined as the ratio of $I_0 - I$ and I_0 , where I and I_0 represent the reductive current of our A-IDA upon with and without the treatment with different concentrations of sarcosine, respectively. Based on this, we further demonstrate that the ratio of current decrease of our sensor was linearly increased with increasing the sarcosine concentration (Fig. 5B) as well as with a dynamic linear within lower concentration range from 0.10 to 2.20 μM ($R = 24.65 + 31.92 C_{\text{sarcosine}} (\mu\text{M})$, $\gamma=0.9953$). More importantly, the proposed A-IDA exhibits rather lower detection limit (30.00 nM, S/N=3), For better comparison of the present work with some of the earlier reports, different methods employed for the detection of sarcosine are enumerated in Table S2. These results strongly indicated that the proposed sensor exhibits high sensitivity and excellent sensing performance toward sarcosine, which could satisfy the requirements of clinical correlative sarcosine monitoring during postoperative and long-term care (1.7 μM) [31]. After the modified electrode was stored in the refrigerator at 4 °C over 2 weeks, no obvious reduction in the response current occurred. These results demonstrate that the electrode exhibits satisfactory reproducibility and stability (As shown in Fig. S11). Furthermore, the selectivity of our A-IDA for sarcosine was investigated upon the addition of different interferences including Fe^{3+} , Cu^{2+} , Glu, Lys, Leu, Trp, His and UA by using amperometric technology. As illustrated in Fig. 5C and Fig. 5D, the signal changes caused by all other interferences are very small, while the sarcosine gives an obvious change of the electrochemical signal, indicating that the proposed sensor has an excellent selectivity for sarcosine determination compared to other amino acids, metal cations and small molecules due to the unique affinity between sarcosine and

NQS molecules. In addition, the applicability and reliability of our A-IDA were further evaluated by using the standard addition method. By spiking standard sarcosine samples with different levels (i.e., 0.5, 1.0, 1.5 μM) in buffer solution, satisfying recoveries were successfully found and presented in Table S1, which also strongly supporting a satisfactory feasibility and applicability of the developed A-IDA in the potential applications of sarcosine determination.

4. Conclusion

In summary, by taking the advantage of indicator displacement assay principle, we have successfully explored an ingenious yet effective strategy for amperometric assay of electrochemically inactive sarcosine by using a naturally organic NQS molecule as the receptor and an electroactive probe of $\text{Ru}(\text{NH}_3)_6^{3+}$ as the indicator. Due to the stronger affinity of the proposed receptor toward sarcosine than toward the indicator species, the developed A-IDA demonstrates highly selective and excellent sensitive performance toward sarcosine sensing purpose. Our finding not only provides opportunities for developing and designing electrochemical inactive and inherent target sensor but also exhibits more benefits as a fundamental innovation insight further for the biomarker determination and resulting disease diagnosis and treatment follow-up.

Supporting Information

Additional information can be seen in Supporting Informations.

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Figure Captions

Fig. 1 The proposed sensing processes of our A-IDA detection to sarcosine.

Fig. 2 (A) The absorption curves of 0.30 mM NQS (a), 0.10 mM $\text{Ru}(\text{NH}_3)_6^{3+}$ (b), 0.15 mM sarcosine (c), a+b (d), a+c (e) and a+b+c (f) in $\text{NaOH-H}_3\text{BO}_3$ buffer solution (pH,10.00) at room temperature, Inset is the photograph of the corresponding substance; Cyclic voltammograms (B) and Differential pulse voltammograms (C) obtained at the proposed $\text{Ru}(\text{NH}_3)_6^{3+}/\text{NQS}/\text{GO}$ modified GCE in 0.10 M KCl before (a) and after (b) immersing into 48.70 μM sarcosine target (pH,10.00). Insert depicts the corresponding electrochemical behaviors of the bare GCE (c), GO(d) and NQS/GO(e) modified GCE at the same condition.

Fig. 3 SEM images of bare GCE (a), GO(b), NQS/GO (c) and $\text{Ru}(\text{NH}_3)_6^{3+}/\text{NQS}/\text{GO}$ (d) modified GCE

Fig. 4 EDS datas of GO(a), NQS/GO(b), $\text{Ru}(\text{NH}_3)_6^{3+}/\text{NQS}/\text{GO}$ modified GCE without (c) and with (d) the introduction of 48.70 μM sarcosine for 10 minutes immersing time.

Fig. 5 (A) DPVs of the $\text{Ru}(\text{NH}_3)_6^{3+}/\text{NQS}/\text{GO}$ modified GCE in 0.10 M KCl after the electrode was immersed into different concentrations of sarcosine for 10 minutes. Concentrations range: 0.10, 0.25, 0.50, 0.74, 1.01, 1.23, 1.75, 2.20, 4.53, 8.80, 16.50, 30.90 and 48.70 μM ; (B) Linear plot of the ratio of current decrease versus sarcosine concentration. Inset is linear plot with lower concentration from 0.10 to 2.20 μM ; (C) Amperometric I-t curve of the $\text{Ru}(\text{NH}_3)_6^{3+}/\text{NQS}/\text{GO}$ modified GCE in 0.10 M KCl after successively immersing in 1 μM sarcosine, FeCl_3 (Fe^{3+}), CuCl_2 (Cu^{2+}), Glutamic (Glu), Lysine (Lys), Leucine (Leu), Tryptophan (Trp), Histidine (His), Uric acid (UA) and 1.50 μM sarcosine. The working potential was -0.4 V; (D) The decreased current within 100 seconds of amperometric I-t curve of the $\text{Ru}(\text{NH}_3)_6^{3+}/\text{NQS}/\text{GO}$ modified GCE in 0.10 M KCl after successively immersing in the sarcosine and different interfering species.

(A)

(B)

Displacement