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Sensitive and selective determination of GSH based on the ECL quenching of Ru(II) 1,10-phenanthroline-5,6-dione complex

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ABSTRACT

Electrochemiluminescence (ECL) material Ru-dpq ($Ru(bpy)_2dpq^{2+}$, dpq=1,10-phenanthroline-5,6-dione; bpy=2,2′-bipyridine) is found to be produced strong and stable anodic ECL signal, which could be quenched by reduced glutathione (GSH) and exhibits high sensitivity and selectivity simultaneously. According to the mass spectra of Ru-SG (product of Ru-dpq reacted with GSH), and single crystal structure of the final oxidized product Ru-dcbpy ($(Ru(bpy)_2dcbpy^{2+}$, dcbpy=3,3-dicarboxy-2,2-bipyridine), we propose a new interacted mechanism between Ru-dpq and GSH. A good linear relation is estimated to be from 0.1 pM to 50 μM in the presence of calcium ion and the detection limit is as low as 0.087 pM (with the signal-to-noise ratio of 3). The relative standard deviation is 2.3% (for three repeated measurements). Furthermore, the ECL signal of Ru-dpq under a constant potential (1.2 V) is extremely stable and the intensity could be maintained over 600 s, which promotes us to determine the concentration of GSH via chronoamperometry.

Introduction

The low molecular weight thiols, widely distributed in the tissues and cells, have been proven to play an important role in metabolism and cellar homeostasis (Kleinman and Richie, 1995; Pelletier and Lucy, 2002). Herein, glutathione is found to be the most abundant cellar thiol and exists in redox equilibrium between sulfhydryl (reduced form, GSH) and disulfide (oxidized form, GSSG) forms (Kizek et al., 2004; Rahman and MacNee, 2000). It has been extensively revealed to be an intermediate in combating oxidative stress and maintaining redox homeostasis that is pivotal for cell growth and function, and its level has been directly linked to some diseases and cancers (Buhl et al., 1989; Kleinman and Richie, 2000; Macdonald et al., 1977).

Various conventional techniques for the determination of GSH and related thiols such as high performance liquid chromatography coupled (HPLC) with different detection methods usually suffer from substantial difficulties in terms of equipment cost, complexity, sample processing, and applicable feasibility in vivo analysis (Amarnath et al., 2003; Chen and Chang, 2004; Guan et al., 2003; Jiang and Ju, 2007; Kawakami et al., 2006; Liu et al., 2005; MacCoss et al., 1999; Wang et al., 2006). Direct electrooxidation of thiol compounds on common solid electrodes limits its selectivity because of the similar oxidation potential of most biological reducing substances with that of thiol (Kuninori and Nishiyama, 1991). Recently, considerable interest has turned to the use of some organic or inorganic electroactive indicators as electron mediators for thiol compounds detection, yet their wide applications are limited by the requirement of finely tuning the experimental conditions for the success of the electrocatalytic reaction between the thiol compounds and the indicator. Thereby, additional efforts are needed to create a more broadly applicable electrochemical approach that would allow sensitive, rapid, and low-cost determination of GSH and related thiols (Wang et al., 2008).

Because of its many unique advantages, such as ultrahigh sensitivity, no light source required and being easy to control (Bard and Faulkner, 2001), electrochemiluminescence (ECL) method has been developed to analysis field, especially Ru(II) compounds. Ru(II) complexes with polypyridyl ligands, such as $Ru(bpy)_3^{2+}$ (Tokel and Bard, 1972), featuring distinctive optical properties and good water solubility contribute significantly to fabricate ideal ECL determination systems. When modified with specific groups, Ru(II) complexes could recognize various analytes, such as $[Ru(bpy)_2(DA\text{-}phen)]^{2+}$ (DA-phen:5,6-diamino-1,10- phenanthroline) reacted with oxynitride to form a “turn-on” structure (Zhang et al., 2013). Ru(II) quinone-containing complexes combined with water molecule to lead “turn on” fluorescence (Poteet and MacDonnell,

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2013). Ru(II) complexes with schiff base cavities could sensing metal cations (Li et al. 2015) and a bimetallic Ru(II) complex could response to anion (Saha et al., 2012). The fluorescence of a ruthenium(II)–copper(II) complex could be quenched by hydrogen sulfide (Ye et al., 2014). Ruthenium(II) with ligand 5-amino-1,10-phenanthroline could be designed to detect apurinic/apyrimidinic endonuclease 1 (Zhuo et al., 2014). In addition, Ruthenium(II) could also triggered the near-infrared luminescence of Nd(III), Yb (III) and Er(III) complexes by Energy-Transfer (Singaravadivel et al., 2013).

However, detection of GSH via ECL of Ruthenium (II) complexes has been reported rarely before (Bertoncello and Forster, 2009; Miao, 2008; Richter, 2004). Several ECL methods about quantum dots, such as single-layer graphene quantum dots (GQDs) and L-cysteine (L-Cys), were found to be able to produce strong cathodic ECL signal as well as could be used to detect GSH (Dong et al., 2014). But the sensitivity is needed to be improved. In addition, its selective sensing is also an intractable issue in analytical and biochemical communities because its detection is interfered by some thiol-containing compounds. In this paper, a Ruthenium (II) complex Ru-dpq was used to detect GSH due to its internal carbonyl group can interact with interfered with sulfydryl in GSH, which caused ECL quenching of Ru-dpq. The signal can hardly be interfered by its disulfide (GSSG) and other cellular compounds, which demonstrates the outstanding characteristics of efficiency, sensitivity and selectivity. Not only the mass spectrum explained the product produced by the reaction of GSH and Ru-dpq, but the consequence of X-ray single crystal diffraction can visually display the molecular structure of the oxidized product as well, which could exhibit the mechanism of ECL quenching process clearly. In a further step, Ru-dpq has an excellent performance in ECL stability. Thus chronoamperometry (CA) was used to study the ECL intensity of Ru-dpq and the determination of GSH content successfully.

Rich GSH content of cancer cells causes their high antioxidant capacity. In this work, the rat breast cancer cell (4T-1) was chosen to determine the GSH content in it. The tested result is approximate to the result detected by GSH kit. The result of GSH content obtained by this work find 3.78 μM in 4T-1 cell lysate, and the content determined via GSH kit is 3.54 μM.

Experimental section

Materials

Tri-n-propylamine (TPrA) was purchased from Alfa Aesar (Tianjin, China). GSH was obtained from Aladdin (Shanghai, China). 4-amino-2,2,6,6-Tetramethypiperidine-1-oxyl free radical (TEMPO) was purchased from Tokyo Chemical Industry (Tokyo, Japan). 4T-1Cell lysate (2.8×10^6 cells) was provided by the West China academy of Pharmacy, Sichuan University. The GSH kit was purchased from Beyotime Biotechnology (Shanghai, China). All the other chemicals were of analytical-reagent grade and used without further purification.

Apparatus

^{1}H NMR measurements were performed with Bruker AVII-600 MHz spectrometer (Switzerland). Mass spectra was obtained with MAT-261 spectrometer (Finnigan Mat, Germany). The CV and ECL curves were recorded simultaneously using a MPI-E electrochemiluminescence analyzer system (Xi'an Remax Analysis Instrument, China). Fluorescence spectra (FL) were measured on Hitachi F-7000 spectrophotometer (Japan) equipped with a 1 cm quartz cell.

Preparation of 1,10-phenanthroline-5,6-dione (dpq)

The following procedure was based on the reported literatures (Paw and Eisenberg, 1997), and it yields the product almost quantitatively. (Fig. S1).

Preparation of (Ru-dpq)Cl_2 and (Ru-dpq)$(PF_6)_2$

121 mg (0.25 mmol) $Ru(bpy)_2Cl_2 \cdot 2H_2O$, 52.5 mg (0.25 mmol) dpq were dissolved in 60 mL dry ethanol, the mixed solution was stirred and refluxed 12 h in nitrogen atmosphere (Chakraborty et al., 2010). The reaction mixture was allowed to cool to room temperature, volume was reduced to 10 mL by rotary evaporation, filtered and to the one half of filtrate added NH_4PF_6 (40.75 mg, 0.25 mmol). The precipitate was separated and washed with water, ethanol and ether successively and then purified by column chromatography using a column packed with silica gel and acetonitrile-10% KNO_3 (9:1) as eluent then obtain the solid (Ru-dpq)$(PF_6)_2$. The single crystal of (Ru-dpq)$(PF_6)_2$ was obtained through ether diffused slowly into the saturated acetonitrile solution of (Ru-dpq)$(PF_6)_2$. Another half of filtrate was purified with the same method as the former one, while used ethanol-10% KNO_3 (9:1) as eluent to gave (Ru-dpq)Cl_2 (Figs. S2 and S3). The purified solid was dried in vacuum at 55 °C for the ECL tests.

Preparation and purification of the products of GSH reacted with Ru-dpq

(Ru-dpq)Cl_2 (0.1 mmol) and GSH (0.1 mmol) were dissolved in 5 mL PBS (pH 7.4), then the mixture was under the same condition of ECL process for 20 min in nitrogen atmosphere. To the mixture NH_4PF_6 (0.2 mmol) was added. The precipitate was separated and washed with ethanol and ether successively. The solid was dried in vacuum at 50 °C and used to perform the mass spectrometry determination. A further purification was performed by column chromatography using a column packed with silica gel (GF-254) and acetonitrile-10% KNO_3 (9:1) as eluent to obtain the solid (Ru-dcbpy)$(PF_6)_2$. The purified solid was also dried in vacuum at 50 °C and used to perform the single crystal diffraction determination (Scheme S2).

The CV and ECL measurements of Ru-dpq

The CV and ECL measurements were conducted at 298 K in a classical three-electrode configuration consisting of a glassy carbon (GC) electrode working electrode, a platinum wire counter electrode, and an Ag/AgCl reference electrode. The GC electrode was polished carefully with 1.0, 0.3, and 0.05 mm Al_2O_3 powders using a fine polishing cloth to obtain a mirror surface, and then ultrasonically cleaned with doubly distilled water and acetone for 5 min respectively. The CV and ECL curves were recorded simultaneously using a MPI-E electrochemical-luminescence analyzer system. The supporting electrolyte was 0.20 M PBS (pH 7.4).

Results and discussion

ECL property of Ru-dpq

The ECL process of Ru-dpq is similar to conventional Ru (II) complexes, as $Ru(bpy)_3^{2+}$, (Miao, 2008; Richter, 2004) which can be enhanced by the co-reaction agent Tri-n-propylamine (TPrA) (Fig. 1, red curve). Notably, after adding GSH, an obvious ECL signal quenching can be observed (Fig. 1, blue curve).

To confirm GSH could react with Ru-dpq directly rather than react with the excited or oxidized state of Ru-dpq, we performed