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The enhanced electrochemiluminescence of lucigenin by some hydroxyanthraquinones

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Abstract

Several hydroxyanthraquinones (emodin, rhein and physcion) were found to strongly enhance the cathodic electrochemiluminescence (ECL) of the lucigenin by scanning under the mode of differential pulse voltammetry. The enhanced intensity was linear with the concentrations of rhein, emodin and physcion. The linear calibration ranges of 8.0×10^{-8} to 2.0×10^{-6}, 3.0×10^{-7} to 8.0×10^{-6} and 1.0×10^{-7} to 1.0×10^{-6} mol/L, the detection limits of 2.1×10^{-8}, 1.6×10^{-7} and 5.2×10^{-8} mol/L were obtained for rhein, emodin and physcion, respectively. Potential-resolved ECL was used to study the possible mechanism of the enhancement effect. An electron transfer pathway was found to be involved in the ECL process. It has been confirmed that the reduced hydroxyanthraquinones reacted with dioxygen to give superoxide radical anion which increased the ECL of the lucigenin. Furthermore, these three hydroxyanthraquinones revealed the different enhanced ECL efficiency in the following order: rhein > physcion > emodin.

Keywords: Electrochemiluminescence; Lucigenin; Hydroxyanthraquinone

1. Introduction

Rhubard is an important Chinese traditional medicine. It has very strong antibacterial action and is used for the treatment of bacterial dysentery. The major active constituents of this herb are hydroxylated anthraquinones, including chrysophanol, emodin, physcion, aloe-emodin and rhein and their glucosides. Hydroxylated anthraquinones are considered to be potential antitumour agents. Especially rhein, emodin and physcion (see Fig. 1) have showed antineuroectodermal tumor activity in vitro and in vivo [1,2]. Therefore, the determination of the hydroxyanthraquinones has been attracted much attention. Previous determination methods of hydroxyanthraquinones were usually based on chemiluminescence (CL) [3], electrochemical detection with mercury and glass carbon electrodes [4], capillary electrophoresis (CE) [5–9] and high-performance liquid chromatography (HPLC) [10–16].

Chemiluminescence (CL) has been used widely to detect many analytes due to its simplicity and high sensitivity. A CL technique for determination of emodin has been reported [3], which is based on the quenching effect of emodin in the CL system of luminol–H_2O_2–Cr^{3+} due to the reaction of emodin and H_2O_2. Electrochemiluminescence (ECL), in contrast to CL, is a process in which light is emitted only when an appropriate voltage is applied to the electrode contacting with the solution containing a proper compound. In most cases, this technique not only has the same advantages of CL analysis, but also performs some other advantages, such as less reagent requirement, more information for mechanism study. However, so far to the best of our knowledge, ECL method has been never applied to determine hydroxylanthraquinones.

The main purpose of this study was to establish a sensitive and rapid ECL method for determination of hydroxylanthraquinones and to explore the possible mechanism of this reaction.

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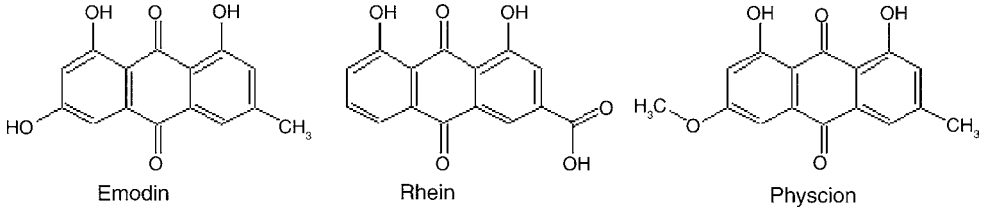

Fig. 1. The molecular structure of the three hydroxyanthraquinones.

2. Experimental

2.1. Chemicals

Lucigenin was purchased from Sigma Chemical Co. (USA) and used without further purification. Rhein, emodin and physcion were purchased from the national institute for the control of pharmaceutical and biological products (Beijing, China). Stock solution of 1.0×10^{-3} mol/L for each hydroxyanthraquinone was prepared and stored at 4 °C in a refrigerator with a tight cover to minimize exposure to light and air. The rhein, emodin and physcion working solutions were made by appropriate dilution with 0.01 mol/L KCl. The Britton–Robinson (B.R.) buffer (pH 2.0–12.0) was prepared by titrating a stock solution containing 0.04 mol/L acetic acid, 0.04 mol/L phosphoric acid, 0.04 mol/L boric acid with 0.2 mol/L sodium hydroxide to the desired pH value.

All other chemicals used in this study were analytical reagent or better. Double-distilled water was used throughout.

2.2. Apparatus

The experimental equipment for ECL measurement including a BPCL ultra-weak chemiluminescence analyzer controlled by a personal computer with BPCL program (Institute of Biophsics, Academia Sinica, China) and a electrochemical analyzer (CHI660a, Shanghai Chenghua instrument Co., China).

A conventional three-electrode system was used as the electrolytic system, which was composed of a glassy carbon electrode as the working electrode, a platinum wire as the counter electrode and Ag/AgCl (saturated KCl) electrode as the reference electrode. A commercial 5 mL cylindroid's glass cell was used as ECL cell. Before each measurement, the working electrode was fixed in the same position and directly faced the window of the pohotomultiplier tube. ECL spectrum was monitored by a 970CRT fluorescence spectrometer (Shanghai, China). The working electrode was pretreated by polishing its surface with aqueous slurries of alumina powders (1.0 and 0.3 μm α-Al_2O_3) on a polishing cloth and then carefully washed with water to give a smooth and clean electrode surface. The ECL cell was washed with 0.2 mol/L nitric acid and water in sequence before use.

2.3. Procedure

A 100 μL of sample solution, 1 mL of 1.0×1.0^{-4} mol/L lucigenin and 1 mL of 0.10 mol/L KCl were added successively to a 10 mL volumetric flask, and then diluted with double-distilled water to required volume. 2.5 mL of the diluted solution was transferred to the ECL cell. A potential between 0.0 and −1.0 V was applied to the working electrode and the ECL signal was recorded simultaneously. The emission at −0.55 V was used to the quantitative analysis, based on the net ECL intensity changes (ΔI, $\Delta I = I_s - I_0$), where I_0 was the background signal (ECL intensity) of the lucigenin system without hydroxyanthraquinones, and I_s was the signal obtained with addition of hydroxyanthraquinones.

3. Results and discussion

3.1. The enhanced ECL of lucigenin by hydroxyanthraquinones

The ECL of lucigenin was primarily examined at a glassy carbon electrode (GCE) by differential pulse voltammetry in neutral aqueous solution. When the applied potential was scanned in the range of 0.0 to −1.0 V, a broad ECL peak of lucigenin was observed (see Fig. 2, curve A). The ECL intensity was greatly enhanced by addition of emodin, physcion or rhein (see Fig. 2, curves B–D). Among these three hydroxyanthraquinones, the highest luminescence intensity was obtained in the presence of rhein, and the lowest in emodin.

3.2. Selection of electrochemical parameters

In our previous work [17], the linear sweep voltammetry (LSV), square wave voltammetry (SWV) and differential pulse voltammetry (DPV) were used to examine the effect of excitation waveform on the ECL of licigenin by using the anodic potential sweep. The results showed that the most stable ECL could be obtained by using DPV.

In this paper, LSV and DPV were selected to examine the effects of electrochemical techniques on this ECL system. The result showed that, when DPV was used, a stronger net ECL intensity was obtained. So DPV mode was selected for the subsequent investigation. To establish the optimal conditions, the luminescent intensity was measured as a function of

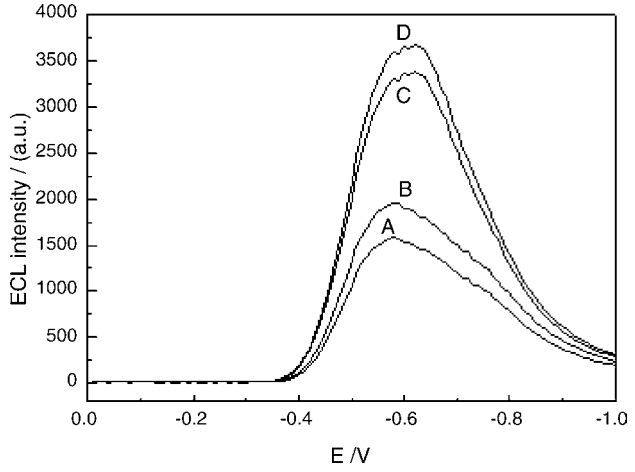

Fig. 2. ECL emission vs. potential scanning under the mode of DPV. (A) 1.0×10^{-5} mol/L lucigenin; (B) 1.0×10^{-5} mol/L lucigenin + 8.0×10^{-7} mol/L emodin; (C) 1.0×10^{-5} mol/L lucigenin + 2.0×10^{-7} mol/L physcion; (D) 1.0×10^{-5} mol/L lucigenin + 2.0×10^{-7} mol/L rhein; [KCl]: 0.01 mol/L; conditions of DPV were the same as in Table 1.

Table 1
Differential pulse voltammetry parameters

Initial E (V)	0.0
Final E (V)	−1.0
Incr. E (V)	−0.004
Amplitude	0.05
Pulse width (s)	0.05
Sampling width	0.0167
Pulse period (s)	0.2

pulse amplitude, pulse width and pulse period. The selected optimum parameters for DPV were showed in Table 1.

Fig. 3 shows the DPVs of lucigenin and anthraquinones in the absence of O_2. It can be seen that the potential range of anthraquinones' cathodic reduction potential (shown in Table 2) was more negative than that of the reduction potential of lucigenin (−0.328 V), which indicated that lucigenin

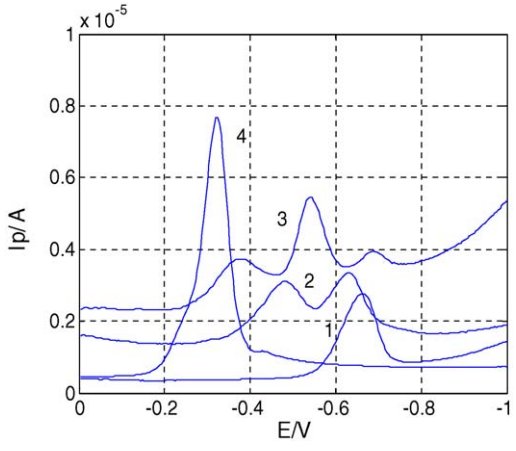

Fig. 3. The DPVs of lucigenin and hydroxyanthraquinones: (1) 1.0×10^{-5} mol/L emodin; (2) 1.0×10^{-5} mol/L physcion; (3) 1.0×10^{-5} mol/L rhein; (4) 1.0×10^{-5} mol/L lucigenin; [KCl]: 0.01 mol/L; conditions of DPV were the same as in Table 1.

Table 2
Cathodic reduction potentials of hydroxyanthraquinones under study

Compounds	E_{c1}	E_{c2}	E_{c3}
Rhein	−0.372	−0.540	−0.688
Physcion	−0.484	−0.632	
Emodin	−0.664		

was in reduced state on the electrode surface when the ECL appeared.

The ECL of lucigenin at Pt electrode has been observed in Zhang's and Qi work [18]. They explained that the ECL peak at −0.36 V was generated from the CL reaction of dissolved oxygen with lucigenin radical cation ($Luc^{\bullet+}$), while the ECL peak at −0.65 V was mainly produced from the CL reaction of reduced lucigenin with electrogenerated H_2O_2 from the dissolved oxygen, and the ECL peak at −0.83 V was generated from the CL reaction of $Luc^{\bullet+}$ with hydroxide anion ($OH^{\bullet}$). In our experiment, there was only one broad ECL peak observed by using glassy carbon electrode as working electrode. The pathway of the major ECL peak is suspected to be generated from the reaction of reduced lucigenin with the electrogenerated $O_2^{\bullet-}$.

3.3. Selection of chemical reaction conditions

3.3.1. Selection of medium

It is well known that medium plays an important role in ECL reaction. The result of the experiment showed that the ECL intensity of lucignein without hydroxyanthraquinones in B.R. buffer was increased with the increasing of pH from 7 to 11, but the enhanced ECL intensity was not increased as expected. The effect of medium on the ECL was examined by using carbonate, phosphate, borate, Tris–HCl, NH_3–NH_4Cl and KCl solution. The highest enhanced ECL intensity was obtained in KCl solution. The effect of the concentrations of KCl on the enhanced ECL was then investigated in detail in the range of 0.0002–0.1 mol/L. The results indicated that the ECL intensity of lucigenin in the absence of hydroxylanthraquinones was increased with the concentration of KCl, while the enhanced ECL intensities changed slightly with the concentration of KCl. When the concentration of KCl was higher than 0.01 mol/L, the background signal was increased greatly. Considering the stabilization and sensitivity of the determination, 0.01 mol/L KCl was used in the following experiments.

3.3.2. Effect of lucigenin concentration

The concentration of lucigenin also effect the ECL intensity. As shown in Fig. 4, the enhanced ECL intensity was increased with the concentration of lucigenin from 2.0×10^{-6} to 1.0×10^{-5} mol/L. But when the concentration of lucigenin was higher than 1.0×10^{-5} mol/L, the blankground was quite high and a significant increase in the noise amplitude of the baseline was observed, which is unfavorable to obtain the higher sensitivity. In addition, higher concentration of lucigenin would lead to more deposition of luc^0 (i.e.

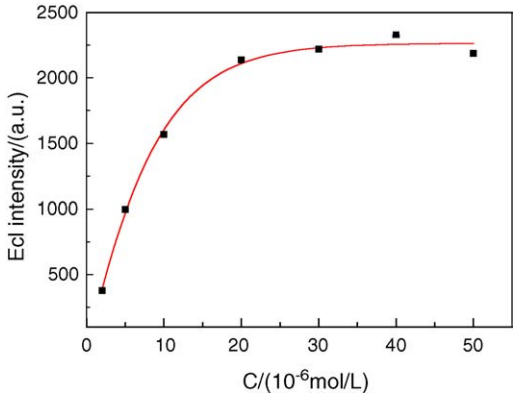

Fig. 4. Dependence of the concentration of lucigenin on enhanced ECL intensity. [Rhein]: 2×10^{-7} mol/L; [KCl]: 0.01 mol/L, [Luc]: 1×10^{-5} mol/L; conditions of DPV were the same as in Table 1.

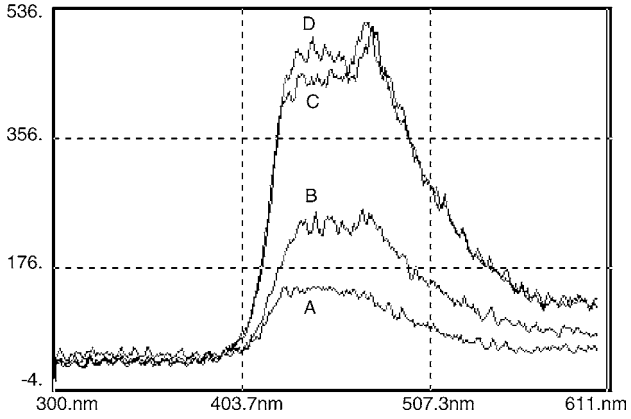

Fig. 5. ECL spectrum of lucigenin in the present or absence of hydroxyanthraquinones: (A) 1.0×10^{-5} mol/L lucigenin; (B) 1.0×10^{-5} mol/L lucigenin + 8.0×10^{-7} mol/L emodin; (C) 1.0×10^{-5} mol/L lucigenin + 2.0×10^{-7} mol/L physcion (D) 1.0×10^{-5} mol/L lucigenin + 2.0×10^{-7} mol/L rhein; [KCl]: 0.01 mol/L; conditions of DPV were the same as in Table 1.

the two-electon reduced form of luc^{2+}, luc = lucigenin) [19] and *N*-methylacridone (NMA) on electrode surface since their solubility in water are small, which would result in the unstabilization of this system. Therefore, 1.0×10^{-5} mol/L lucigenin was selected for all experiments.

3.4. Interference

In order to apply the proposed method to the determination of hydroxyanthraquinones in pharmaceutical samples, the interference of some possible co-existing ions was examined. A foreign ion was not considered to interfere if it caused a relative error less than 5% during the determination of 6.0×10^{-7} mol/L hydroxyanthraquinones. The tolerated ratio of foreign substances to 6.0×10^{-7} mol/L hydroxyanthraquinones was1000 for Na^+, K^+, Cl^-, Ac^-, SO_4^{2-}, NO_3^-; 500 for Mg^{2+}, Glucose, lactose; 200 for amylum, Ca^{2+}, Ba^{2+}, NH_4^+; 5 for Co^{2+}, Fe^{3+}, Mn^{2+}; 2 for Pb^{2+}, Cu^{2+}, Cd^{2+}; 1 for Cr^{3+}.

Suitable amount of EDTA must be added to avoid the interference from metal ions because of the formation of stable complexes of metal ions with EDTA.

In addition, since several hydroxyanthraquinones always co-exist, a separated technique, such as CE should be coupled with ECL for practical analytical application.

3.5. Linear response range, detection limit and precision

Working curves were obtained under the optimized experimental condition, and the results are shown in Table 3. The regression equations revealed a linear relationship between the concentration of hydroxylanthraquinones and the net intensity of ECL. The relative standard deviations ($n = 11$) were 3.1, 2.7 and 2.1% for determining 2.0×10^{-7} mol/L of rhein, 2.0×10^{-6} mol/L of emodin and 2.0×10^{-7} mol/L of physcion, respectively.

3.6. Mechanism

It is known that the pathway of the ECL of lucigenin is due to its reaction with the electrogenerated oxygen species [20].

$$Luc^{2+} + e = Luc^{\bullet +}$$

$$Luc^{\bullet +} + O_2^{\bullet -} = NMA^* \rightarrow NMA + h\nu.$$

We also found that the ECL peak almost disappeared when the solution was deaerated with nitrogen either in the absence or presence of hydroxyanthraquinones. Thus, the scheme is closely related to the dissolved oxygen. In order to elucidate the processes of this system, the ECL spectrum of lucigenin (see Fig. 5) was obtained when a potential of −0.60 V versus Ag/AgCl was applied. As shown in Fig. 5, the maximum wavelength of the ECL emission was 470 nm in the absence or presence of hydroxyanthraquinones. These results suggested that the major emitter was *N*-methylacridone (NMA^*), the reduction product of lucigenin. Some of the NMA^* can also transfer their energy to lucigenin to form exicted luci-

Table 3
Linear relationships of chemiluminescence intensity with the concentration of estrogens

	Agents linear range (mol/L)	Linear regression equation (*C*, mol/L)	Correlation coefficient (*r*)	Detection limit (*S*/*N* = 3, mol/l)
Rhein	8.0×10^{-8}–2.0×10^{-6}	$I_{ECL} = 1725.9C + 696.9$	0.9916	2.1×10^{-8}
Emodin	3.0×10^{-7}–8.0×10^{-6}	$I_{ECL} = 179.54C - 13.854$	0.9934	1.6×10^{-7}
Physcon	1.0×10^{-7}–1.0×10^{-6}	$I_{ECL} = 4667.5C - 292.14$	0.9959	5.2×10^{-8}

I_{ECL} is the net intensity of ECL. The relative standard deviations ($n = 11$) were 3.1% for determination of 2.0×10^{-7} mol/L rhein, 2.7% for 2.0×10^{-6} mol/L emodin and 2.1% for 2.0×10^{-7} mol/L physcion.

genin. Both the excited lucigenin and NMA* species return to their ground states simultaneously and give out a mixed broad light emission peak from 420 to 580 nm with maximum wavelength at about 470 nm.

Based on the results of our experiments and the previous reports [21,22], the mechanism of the ECL can be proposed. The hydroxyanthraquinones powerfully enhanced the ECL of lucigenin due to their abilities to produce superoxide anion radicals ($O_2^{\bullet -}$) and their conjugated acid, the hydroperoxyl radical ($HOO^{\bullet}$). It is well known that hydroxyanthraquinones are good antioxidants and it is reasonable to assume a reaction of hydroxyanthraquinones with molecular oxygen. The reaction following two-electron reduction of dihydroxyanthraquinone derivatives involves electron and proton transfer from semiquinone anion radical intermediate to molecular oxygen to form deprotonated hydroxyanthraquinones and freshly superoxide species [21].

The influence of the substitution on the quinone ring is interesting. Our studies revealed a strong relationship between substituent effect and reduction potential. This relationship is correlative with the enhanced luminescence. It is well known that –COOH is more electro-withdrawing than –OH or $–OCH_3$, –COOH group causes a shift of reduction potential to more positive value and shows higher enhanced ECL efficiency, whereas –OH (hydroxy) and –OMe (methoxy) cause negative effect [23] and show lower enhanced ECL efficiency. Because of the lower electron-withdrawing property of hydroxyl, emodin which contains a hydroxy group presents a shift to more negative values and lower enhanced ECL efficiency than physcion which has a methoxy group.

4. Conclusions

Based on the ability of hydroxyanthraquinones to enhance the ECL of lucigenin, an ECL method has been developed for determination of hydroxyanthraquinones in a neutral aqueous solution in this paper. Comparing with the traditional method, the ECL system described in this paper was simple, and the operation was rapid and convenient. The detection limit of the proposed method is lower than those reported in the previous literatures. The ECL reaction could be achieved in neutral solution and need no specific oxidizing reagent, which provides a friendly condition for biomolecules.

In addition, the possible mechanism of the system was discussed in detail. It was proposed that *N*-methylacridone (NMA) was the emitter of this ECL reaction, and the enhancement effect of hydroxyanthraquinones results from the reaction of $Luc^{\bullet +}$ and the superoxide anion radicals ($O_2^{\bullet -}$) or the hydroperoxyl radical ($HOO^{\bullet}$) which produced in the process of the electrochemical reduction of hydroxyanthraquinones. We also found the enhanced ECL efficiency is relative to the substitution on the quinone ring. Electron-withdrawing substituent shows higher the enhanced ECL efficiency than electron-donating substituent.

Acknowledgements

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