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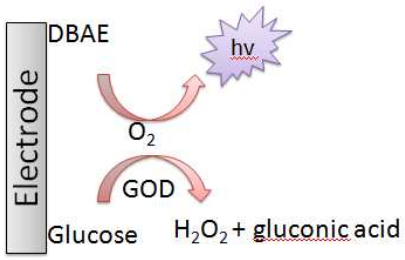

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ARTICLE TYPE

Strong Anodic Electrochemiluminescence from Dissolved Oxygen with 2-(Dibutylamino) ethanol for Glucose Oxidase Assay

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A strong anodic electrochemiluminescence of dissolved oxygen with 2-(dibutylamino) ethanol is observed and investigated in aqueous solution for the first time. Benefiting from its strong anodic electrochemiluminescence emission, an oxygen-responsive electrochemiluminescence system is developed to detect glucose oxidase.

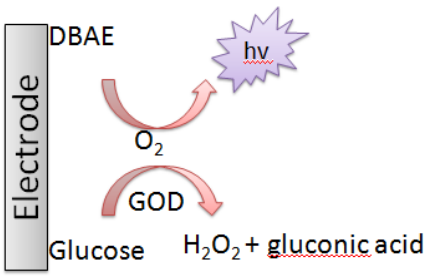
Electrochemiluminescence (ECL), a sort of luminescence produced during electrochemical reactions in solutions, has been extensively studied and widely applied in many fields owing to its versatility, simplified optical setup, very low background signal, good temporal and spatial control.[1, 2] The ECL techniques possess the advantages of sensitivity and specificity. After a long journey of almost half a century, it has been proved to be a useful analytical technique for various analytes such as immune molecules, proteins, DNA oligonucleotides, enzyme inhibitors, cells and some small molecules. [3-10]

In the past decades, many researchers are thriving for new luminophores, applications, fundamental analysis of its mechanisms, making its instrumentation easier, and achieving lower limit of detection. Among various luminophores including $Ru(bpy)_3^{2+}$, luminol, quantum dots and etc., $Ru(bpy)_3^{2+}$- tri-*n*-propylamine (TPrA), luminol-H_2O_2, and QDs-$S_2O_8^{2-}$ have been thoroughly investigated and widely used in analysis.[11,12] Although oxygen has been observed involving all of the above ECL processes, its ECL was underestimated and ignored. In most of cases, it was taken as a background due to its low intensity, and no more attention has been paid to explore its further applications. Recently, Kumar and Bard reported that the background ECL emission at 630 nm during the electrochemical oxidation of TPrA in the presence of dissolved O_2 in acetonitrile solution can be attributed to the dimeric $^1\Delta_g$ state of O_2. [13]

2-(Dibutylamino)ethanol (DBAE) is a recently used coreactant in ECL sensors. Liu and co-workers found that DBAE is more environment friendly and promising strong ECL coreactant in comparison with the widely used TPrA for $Ru(bpy)_3^{2+}$ ECL.[14] They reported that the ECL intensity of the $Ru(bpy)_3^{2+}$/DBAE system at Pt electrode was approximately 100 times stronger than that of the widely used $Ru(bpy)_3^{2+}$/TPrA system, when 25 mM of each coreactant was used in 0.1 M phosphate buffer (pH7.5). From then on, many ECL sensors has been developed based on the $Ru(bpy)_3^{2+}$/DBAE system, e.g., the quantitative determination of tetracycl and dopamine.[15,16] In addition, DBAE has also acted as the coreactant for other luminophores, such as superradiant organic dye J-aggregates and quantum dots.[17-19]

Glucose oxidase (GOD) is a flavin enzyme that was first discovered by Muller (1928) in Aspergillus nigerextracts.[20,21] This enzyme has gained wide application in several industries such as food preservative and color stabilizer,[22] the production of gluconic acid and the textile industry.[23-25] Furthermore, many glucose biosensors based on the interaction between GOD and glucose by different techniques have been fabricated,[26-31] which have potentially been used to monitor the blood glucose levels in diabetics.

In this work, we observed that the electro-oxidation of DBAE in the presence of oxygen can produce a strong ECL signal in aqueous solution without any other luminophore, like $Ru(bpy)_3^{2+}$. The ECL signal of O_2 + DBAE system was much stronger than that of the O_2 + TPrA system at a Pt electrode. Hence, we proposed a simple method for the detection of GOD with high selectivity and sensitivity by using the ECL intensity change of dissolved oxygen with DBAE system. The detection mechanism is shown in Scheme 1.



Scheme 1 Schematic illustration of GOD detection based on O_2 + DBAE ECL system.

As shown in Scheme 1, the oxidation of glucose catalyzed by GOD can generate hydrogen peroxide and gluconic acid in the presence of oxygen.[32] With the consumption of oxygen, the ECL intensity of O_2 + DBAE system decreased gradually. Thus, the quantitative determination of GOD can be carried out according to the change of the ECL intensity. To the best of our knowledge, the application of O_2 + DBAE system to detect GOD has never been reported before.

To achieve the determination of GOD, all electrochemical and ECL experiments were carried out in a three-electrode cell with

a platinum working electrode (Pt, diameter 3 mm), a Pt wire counter electrode and an saturated Ag/AgCl electrode as reference electrode, as shown in Figure 1. The working electrode was fixed through a hollow screw towards the bottom of the three-electrode cell, which is transparent quartz glass window. The ECL intensities were measured through the quartz glass window by the photomultiplier tube (PMT) of MPI-E ECL Analyzer (Xi'an Remax Analysis Instrument Co. Ltd.). The PMT was biased at 900 V in the experiments. Chemicals and solutions, the physical images of the ECL cell and the detection equipment are shown in the supporting information.

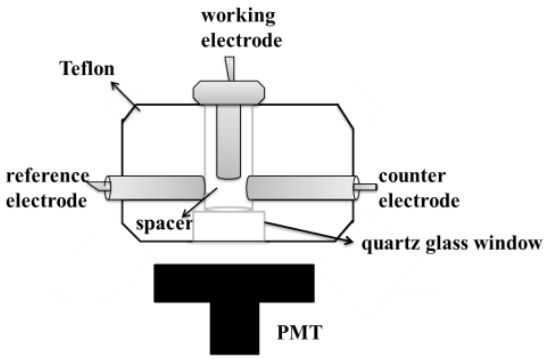


Fig. 1 Diagram of the sealed three-electrode cell on PMT.

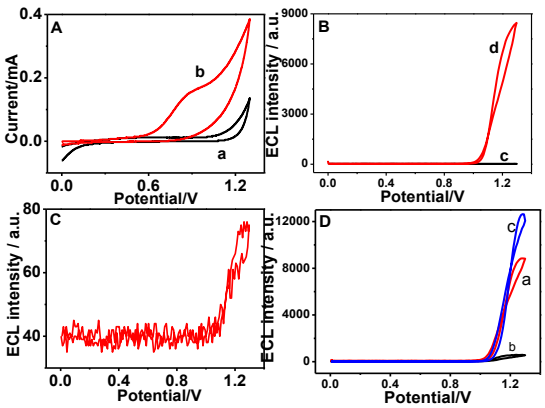


Fig. 2 A) CVs of Pt electrode in air-saturated PBS without (a) and with (b) 90 mM DBAE. B) The corresponding ECL vs. potential curves of Pt electrode in air-saturated PBS without (c) and with (d) 90 mM DBAE. C) The ECL intensity vs. potential curve of Pt electrode in air-saturated PBS with 90 mM TPrA. D) The ECL intensity vs. potential curves of Pt electrode in (a) air-saturated, (b) nitrogen-saturated and (c) oxygen-saturated PBS with 90 mM DBAE. PBS (0.1 M, pH 7.4); Scan rate: 100 mV s^{-1}.

Figure 2A and 1B are the cyclic voltammograms (CVs) and the corresponding ECL intensities on a Pt electrode in the absence and presence of 90 mM DBAE in 0.1 M PBS (pH 7.4), respectively. As expected, no obvious Faradic current (Fig.2A, Curve a) and no ECL signal (Fig.2B, Curve c) were observed in the absence of DBAE during a potential scan range between 0 and 1.2 V. Nevertheless, after 90 mM DBAE was added into the above solution, a typical irreversible anodic peak is emerged as shown in Figure 2A (Curve b), which is attributed to the oxidation of DBAE. Meanwhile, the corresponding ECL intensities increase dramatically as shown in Figure 2B (Curve d).The onset of luminescence occurs near 1.0 V, and then the ECL intensity rises up steeply from 1.0 V until it reaches a maximum near 1.2 V. Furthermore, the ECL of O_2 + TPrA system was also performed and shown in Figure 2C. The ECL intensity obtained at O_2 + DBAE is almost 250 fold larger than that at O_2 + TPrA system.

To further elucidate the observed ECL mechanism, the ECL intensities vs. potential curves of Pt electrode in air-saturated (Fig.2D, Curve a), N_2-saturated (Fig.2D, Curve b) and O_2-saturated (Fig.2D, Curve c) PBS with 90 mM DBAE were performed and compared. As shown in Figure 2D, after the N_2 bubbled through the air-saturated solution for 15 minutes, the ECL intensity of the N_2-saturated solution becomes very small. However, the ECL intensity rises after the O_2 bubbled for 15 minutes. This suggests that the oxygen participated in the reaction. According to the reaction mechanism of O_2+TPrA system mentioned previously,[13] we suppose the ECL mechanism of O_2 + DBAE system as follows,

$$DBAE \rightarrow DBAE^{\bullet+} + e^- \quad (1)$$

$$DBAE^{\bullet+} \rightarrow DBAE^{\bullet} + H^+ \quad (2)$$

$$DBAE^{\bullet} + O_2 \rightarrow DBAE^{+} + O_2^{\bullet-} \quad (3)$$

$$DBAE^{\bullet+} + O_2^- \rightarrow DBAE + {}^1O_2 \quad (4)$$

$$2\,{}^1O_2 \rightarrow ({}^1O_2)_2^* \quad (5)$$

$$({}^1O_2)_2^* \rightarrow 2\,{}^3O_2 + h\nu \quad (6)$$

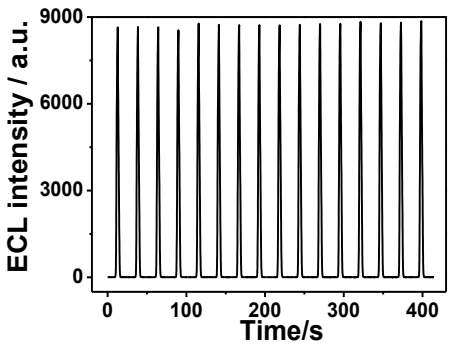


Fig. 3 ECL responses of the O_2 + DBAE system obtained during a continuous potential scan on Pt electrode in PBS (0.1 M, pH 7.4). Scan range: 0 to +1.3 V. Scan rate: 100 mV s^{-1}.

The ECL behavior of O_2 + DBAE system at Pt electrode was quite stable. As shown in Figure 3, under continuously cyclic potential scanning for 16 cycles in 0.1M PBS (pH 7.4) containing 90 mM DBAE, the ECL intensities show no obvious changes. Hence, this system with high ECL intensity could be suitable for further applications in anodic ECL analysis.

In order to obtain a high sensitive sensor for the detection of GOD, the optimization of the conditions is essential. Firstly, the effect of the concentration of DBAE was investigated and optimized. The anodic ECL response depends on the concentration of DBAE was shown in Figure 4A. For various amounts of DBAE, the ECL intensities in the air-saturated detection solution gradually rise with the increasing concentration and tend to be stable after the concentration more than 90 mM. Therefore, 90 mM is chosen as the optimum concentration of DBAE for the next ECL testing solution.

The ECL intensity of O_2 + DBAE system is highly dependent

on the solution pH. Hence, the ECL intensity of the dissolved oxygen with 90mM DBAE in PBS was tested at different pH values. As shown in Figure 4B, the ECL intensity was greatly enhanced with the increase of pH value in the range of 5.0 to 9.0. The ECL signal of O_2 + DBAE system is weak in acidic media, although the solubility of DBAE in acidic media is greater than alkaline media, which can be attributed to the protonation of amines retards their oxidation. However, the bioactivity of GOD was much higher in acidic media. Hence, pH 7.4 was chosen as the optimum pH value in the following experiments.

The effect of the amount of hydrogen peroxide to the O_2 + DBAE system was also investigated because H_2O_2 is the product of GOD and glucose in the presence of oxygen. Figure 4C shows that the concentration of hydrogen peroxide had an effect on the ECL intensity of O_2 + DBAE system. After a high concentration of H_2O_2 (e.g. 1mM) was manually added into the ECL testing system, the anodic ECL intensity displayed an obvious decrease, which may be due to oxidation of DBAE by hydrogen peroxide. Hydrogen peroxide was generated in our experiment, which could make the test of enzyme more sensitive.

Furthermore, the effect of the reaction time on the ECL intensity of O_2 + DBAE + glucose + GOD system was studied. As shown in Figure 4D, after the addition of GOD to the mixed solution, the ECL intensity decreased rapidly in the range of 1 to 5 min, then changed slowly. This result indicates that the reaction between glucose and GOD is rapid. Hence, we chose 5 min as the optimum reaction time in the following ECL experiments.

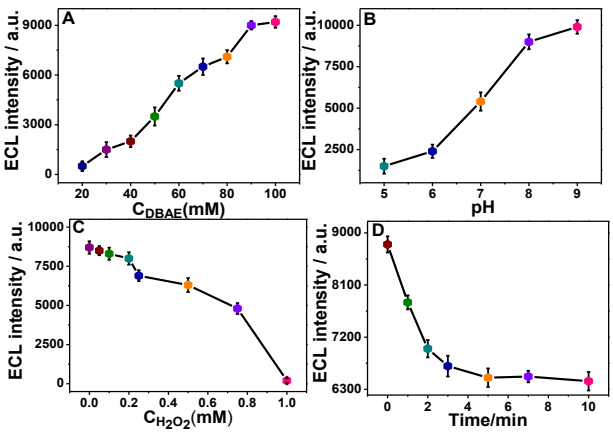


Fig. 4 A) The relation between the ECL intensity and concentration of DBAE. B) Effect of pH on the ECL intensity of O_2 + DBAE system. C) The influence of H_2O_2 concentration on the ECL intensity of O_2 + DBAE system. D) Effect of reaction time on the ECL intensity of O_2 + DBAE + glucose + GOD system. Concentrations: 90 mM DBAE, 10 mM glucose, 10 μg mL^{-1} GOD. ECL detection buffer: PBS (0.2 M, pH 7.4). Scan range: 0 to +1.3 V. Scan rate: 100 mV s^{-1}.

The O_2 + DBAE enhanced ECL system was firstly utilized to roughly monitor the concentration of oxygen in aqueous medium. To remove as much dissolved oxygen as possible prior to making measurements, a test solution containing 90 mM DBAE was degassed with N_2 for 15 min. The steady-state ECL intensity of N_2-saturated solution was measured (Figure 5, Curve a), and then the air-saturated solution containing about 0.25 mM O_2 with 90 mM DBAE was gradually added to the above solution and stirred to ensure a well-mixed solution. As shown in Figure 5, the ECL intensities of O_2 + DBAE system gradually increased with the increasing concentrations of O_2. From the inset of Figure 5, the ECL intensity had good linearity with the concentration of O_2 in the range from 4 to 27 μM (R^2 = 0.997).

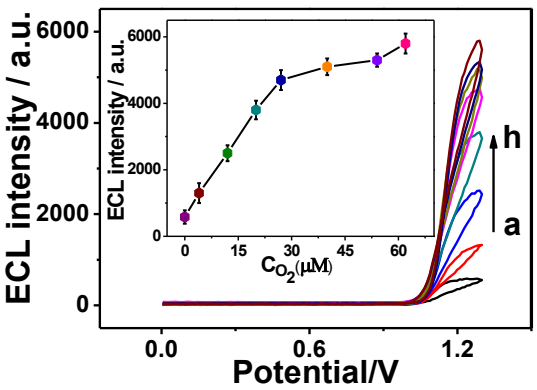


Fig. 5 The ECL intensity vs. potential curves of O_2 + 90mM DBAE system on Pt electrode with different concentrations of O_2 (from a to h: 0, 4, 12, 20, 27, 40, 54, 62 μM, respectively). Inset: the plot of ECL intensity vs. the concentration of O_2. ECL detection buffer: PBS (0.1 M, pH 7.4). Scan rate: 100 mVs^{-1}.

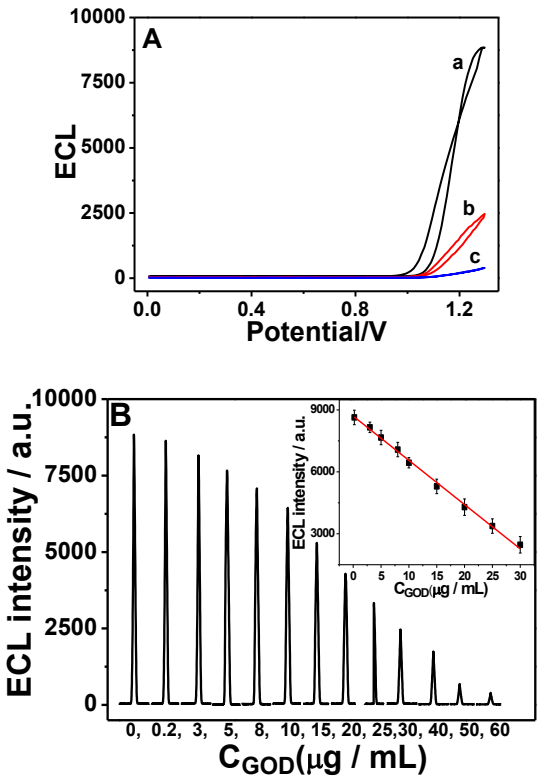


Fig. 6 A) The ECL intensity vs. potential curves of (a) O_2 + 90mM DBAE, (b) O_2 + 90mM DBAE with 30 μg mL^{-1} GOD and (c) with 60 μg mL^{-1} GOD on Pt electrode in PBS (0.2M, pH 7.4). B) The ECL curves of O_2 + 90mM DBAE on Pt electrode with different concentrations of GOD (from left to right, 0.2, 3, 5, 8, 10, 15, 20, 25, 30, 40, 50, 60 μg mL^{-1}, respectively) in PBS (0.1M, pH 7.4). Scan rate, 100mV s^{-1}. Inset: the plot of ECL intensity vs. the concentration of GOD.

To detect GOD, the experiments were carried out in a sealed cell with the volume of 300 μL of PBS (0.1 M, pH7.4) containing 90 mM DBAE as coreactant and 10 mM glucose as substrate. The addition of GOD catalyzing the oxidation of glucose consumes the dissolved oxygen in the solution, thus induces the decreasing of ECL intensity of O_2 + DBAE system. The ECL intensity of the mixed solution was measured and recorded after the introduction of GOD for 5 min incubation in the cell. Figure 6A exhibited the ECL behaviors of the O_2 + DBAE system after the addition of GOD of 30 (Curve b) and 60 μg mL^{-1} (Curve c), respectively. It is obvious to find that the ECL intensity decreased (Curve b and c) after the introduction of GOD. Figure 6B displayed the resulting ECL responses to various concentrations of GOD and the inset of Figure 6B exhibited the corresponding derived calibration curve relating to the concentration of GOD in the range from 0.2 to 30 μg mL^{-1} (R^2 = 0.996).The actual determined limit is 0.05 μg mL^{-1}. Meanwhile, metal ions and anions including Ca^{2+}, K^+, Na^+, Mg^{2+}, Zn^{2+}, Br−, ClO_4^-, Cl^-, NO_3^-, PO_4^{3-}, SO_4^{2-}, other proteins such as bovine serum albumin, protamine, amino acids such as DL-Aspartic acid, L-Histamine, L-lysine did not influence the determination of GOD even if they were kept at a concentration 100 times higher than that of GOD due to the specificity of enzyme catalysis of glucose oxidation by consumption of oxygen.

In summary, a strong anodic ECL of dissolved oxygen with DBAE was obtained and investigated in this work. Based on the anodic ECL of O_2 + DBAE system, a novel anodic ECL biosensor for the detection of GOD has been developed. Apparently, the biosensing protocol can be also suitable for other biologically consumption of oxygen bio-systems. Furthermore, the promising properties of such ECL biosensing concept may be useful for the development of immunesensors or DNA sensors involving a target labeled by an oxidase.

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Notes and references

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