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Effect of hydroxyl and amino groups on electrochemiluminescence activity of tertiary amines at low tris(2,2′-bipyridyl)ruthenium(II) concentrations

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ABSTRACT

ECL of several amines containing different numbers of hydroxyl and amino groups was investigated. N-butyldiethanolamine is found to be more effective than 2-(dibutylamino)ethanol at gold and platinum electrodes, and is the most effective coreactant reported until now. Surprisingly, ECL intensities of monoamines, such as 2-(dibutylamino)ethanol and N-butyldiethanolamine, are much stronger than that of diamines including N,N,N′,N′-tetrakis-(2-hydroxyethyl)-ethylenediamine and N,N,N′,N′-tetrakis-(2-hydroxypropyl)ethlenediamine. The striking contrast between ECL signals of the investigated monoamines and diamines may result from more significant side reactions of diamines, such as the intramolecular side reactions between oxidative amine cation radicals and reductive amine free radicals.

1. Introduction

Tris(2,2′-bipyridyl)ruthenium(II) ($Ru(bpy)_3^{2+}$) electrochemiluminescence (ECL) has received much attention [1,2]. Its analytical applications can be roughly divided into two categories [3–6]. One involves $Ru(bpy)_3^{2+}$ ECL immunoassays where $Ru(bpy)_3^{2+}$ concentrations are much lower than coreactant concentrations [7–11]. The other involves coreactant determination where $Ru(bpy)_3^{2+}$ concentrations are generally higher than coreactant concentrations [12–26]. Much effort has been made to investigate the relationships between the ECL efficiency and coreactant structures under the condition of the later category, and several valuable general conclusions have been drawn [14,15,27].

Recent studies show that $Ru(bpy)_3^{2+}$ ECL can be generated through several routes, such as catalytic oxidation route, direct oxidation route, and the route involving reaction between $Ru(bpy)_3^{+}$ and amine cation radicals [28–40]. The role of these routes is closely related to the relative concentrations between $Ru(bpy)_3^{2+}$ and amines. The direct oxidation route becomes important as the amine concentrations increase and the $Ru(bpy)_3^{2+}$ concentrations decrease. These significant discoveries indicate that the relationship between the ECL efficiency and amine structures may change as the relative concentrations between $Ru(bpy)_3^{2+}$ and amines change. Therefore, it is important to investigate the relationship when $Ru(bpy)_3^{2+}$ concentrations are much lower than that of amines in search of better coreactants for $Ru(bpy)_3^{2+}$ ECL immunoassays.

Enlightened by the above studies, we investigated ECL of several monoamines and diamines containing various numbers of 2-hydroxyethyl or 2-hydroxypropyl when the $Ru(bpy)_3^{2+}$ concentrations are much lower than that of amines. The investigation shows that the monoamines exhibit much stronger ECL intensity than diamines, electron-withdrawing hydroxyethyl can dramatically increase ECL activity through promoting the oxidation of amines [39], and N-butyldiethanolamine is the most efficient coreactant so far.

2. Experimental

2.1. Chemicals

Tri(2,2′-bipyridyl)ruthenium(II) dichloride hexahydrate, 2-(dibutylamino)ethanol, and N-butyldiethanolamine were purchased from Aldrich. N,N,N′,N′-tetrakis-(2-hydroxyethyl)-ethylenediamine (HEEDA) and N,N,N′,N′-tetrakis-(2-hydroxypropyl)ethlenediamine (HPEDA) were purchased from ACROS Organics and Tokyo Chemical Industry Co. Ltd., respectively (Fig. 1).

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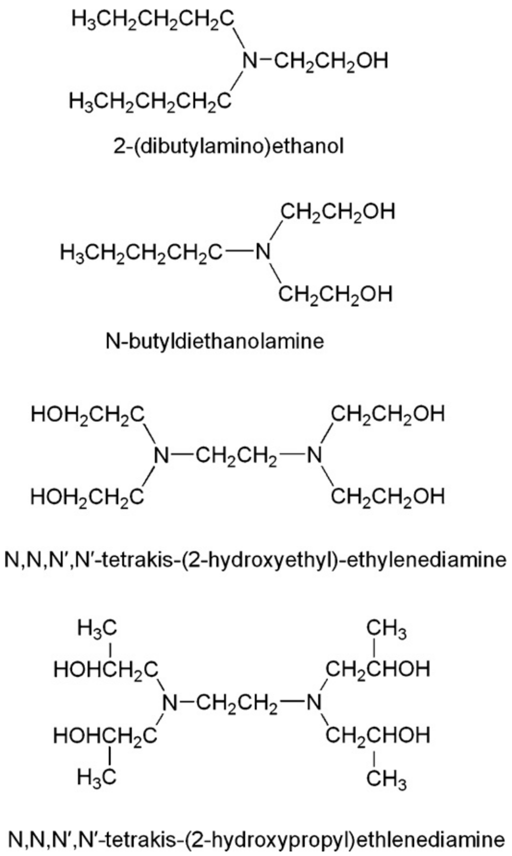

Fig. 1. Structures of 2-(dibutylamino)ethanol, N-butyldiethanolamine, HEEDA, and HPEDA.

2.2. Apparatus

Electrochemical measurements were carried out in a conventional three-electrode cell with a CHI 800B. A gold electrode, platinum electrode, or glassy carbon electrode were employed as working electrodes (3 mm in diameter). An Ag/AgCl (saturated KCl) electrode and a gold wire were used as the reference electrode and the auxiliary electrode, respectively. The ECL signals (I_{ECL}) were measured with a BPCL-1-KIC luminescence analyzer, and the photomultiplier tube was biased at 700 V.

3. Results and discussion

3.1. ECL and electrochemistry at gold electrodes

Fig. 2 shows the dependence of ECL intensity on the concentrations of N-butyldiethanolamine, HEEDA, and HPEDA. The maximum ECL intensity of N-butyldiethanolamine is obtained at a concentration of 15 mM. An earlier report showed that the maximum ECL intensity of 2-(dibutylamino)ethanol was obtained at a concentration of 20 mM [39]. The maximum ECL intensity of the $Ru(bpy)_3{}^{2+}$/N-butyldiethanolamine system is about 1.24 times that of the maximum ECL intensity of the $Ru(bpy)_3{}^{2+}$/2-(dibutylamino)ethanol system if the concentration of $Ru(bpy)_3{}^{2+}$ is 1 μM. The comparison indicates that ECL intensity depends significantly on the numbers of hydroxyethyl, and N-butyldiethanolamine is the most efficient coreactant reported so far. Surprisingly, both HEEDA and HPEDA containing two tertiary amino groups exhibit much weaker ECL intensities than N-butyldiethanolamine.

The linear sweep voltammograms of these amines were studied to explain the effect of the substituents on ECL intensities. The direct oxidation of N-butyldiethanolamine occurs at about 0.9 V, and the anodic current is larger than that of 2-(dibutylamino)ethanol (Fig. 3). This suggests that two hydroxyethyl groups promote the amine oxidation more effectively than one, and thus increase the ECL intensity. The importance of N-butyldiethanolamine oxidation was further confirmed by the following two experimental results. First, both the ECL intensity and the anodic current (at 0.9 V) vary linearly with the square root of the scan rate ($\nu^{1/2}$) and the anodic current from the oxidation of $Ru(bpy)_3{}^{2+}$ is negligible (Fig. 3 inset), demonstrating that the ECL process is related to the direct oxidation of N-butyldiethanolamine [34]. Second, the lower $Ru(bpy)_3{}^{2+}$ concentration, the larger ratio of ECL intensity of the $Ru(bpy)_3{}^{2+}$/N-butyldiethanolamine system to that of the $Ru(bpy)_3{}^{2+}$/2-(dibutylamino)ethanol system. For example, the ECL intensity of the $Ru(bpy)_3{}^{2+}$/N-butyldiethanolamine system at gold electrodes is greater than that of the $Ru(bpy)_3{}^{2+}$/2-(dibutylamino)ethanol system when $Ru(bpy)_3{}^{2+}$ is 1 μM, and is close to that of the $Ru(bpy)_3{}^{2+}$/2-(dibutylamino)ethanol system when $Ru(bpy)_3{}^{2+}$ is 1 mM.

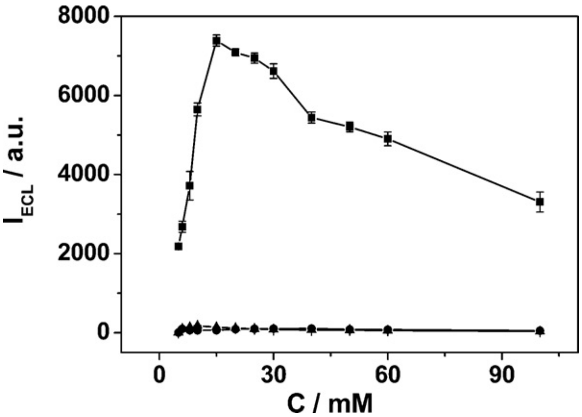

Fig. 2. Dependence of the ECL peak intensity on the concentrations of N-butyldiethanolamine (■), HEEDA (●), and HPEDA (▲) at the gold electrode in 0.1 M PBS containing 1 μM $Ru(bpy)_3{}^{2+}$. The potential was stepped from 0 to 1.35 V. Each point is an average of three successive measurements.

Both diamines HEEDA and HPEDA show significantly larger anodic currents and weaker ECL intensities than N-butyldiethanolamine and 2-(dibutylamino)ethanol (Fig. 3). The observation is probably attributed to more significant side

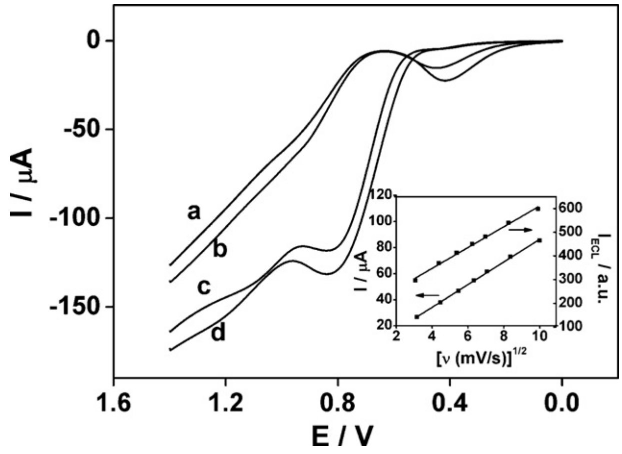

Fig. 3. Linear sweep voltammograms at the gold electrode in 0.1 M PBS containing 1 μM $Ru(bpy)_3{}^{2+}$ and (a) 15 mM 2-(dibutylamino)ethanol, (b) 15 mM N-butyldiethanolamine, (c) 7.5 mM HEEDA, and (d) 7.5 mM HPEDA. Scan rate: 0.1 V/s. Inset: the linear relationship of N-butyldiethanolamine between the ECL intensity or the anodic current and the square root of the scan rate ($\nu^{1/2}$).

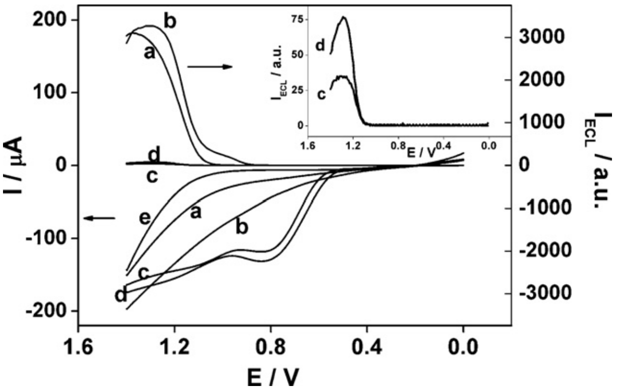

Fig. 4. Linear sweep voltammograms and ECL curves at the platinum electrode in 0.1 M PBS containing 1 μM $Ru(bpy)_3^{2+}$ and (a) 15 mM 2-(dibutylamino)ethanol, (b) 15 mM N-butyldiethanolamine, (c) 7.5 mM HEEDA, and (d) 7.5 mM HPEDA. The (e) line represents the data in 0.1 M PBS. Scan rate: 0.1 V/s. Inset: ECL curves of HEEDA and HPEDA.

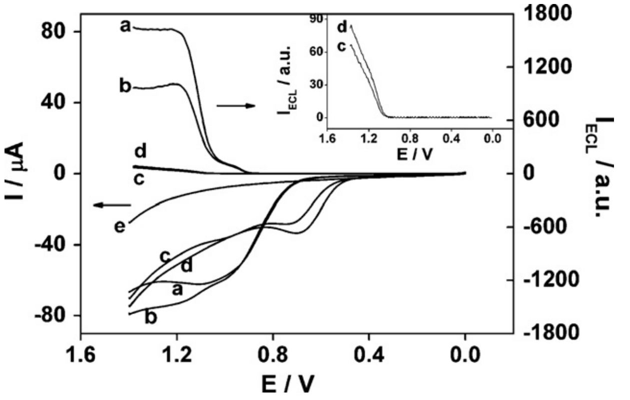

Fig. 5. Linear sweep voltammograms and ECL curves at the glassy carbon electrode in 0.1 M PBS containing 1 μM $Ru(bpy)_3^{2+}$ and (a) 3 mM 2-(dibutylamino)ethanol, (b) 3 mM N-butyldiethanolamine, (c) 1.5 mM HEEDA, and (d) 1.5 mM HPEDA. The (e) line represents the data in 0.1 M PBS. Scan rate: 0.1 V/s. Inset: ECL curves of HEEDA and HPEDA.

reactions of diamines. For example, previous studies of the ECL mechanism (Eqs. (1)–(5)) show that the oxidation of amine can genernate oxidative amine cation radicals and reductive amine free radicals (Eq. (2)). It is likely that the oxidation of the diamines may generate intermediates that contain both oxidative amine cation radicals and reductive amine free radicals. The intramolecular side reaction between oxidative amine cation radicals and reductive amine free radicals (Eqs. (6) and (7)) may waste the ECL reaction intermediates, resulting in weaker ECL despite larger anodic currents [34]:

$$Ru(bpy)_3{}^{2+} - e \rightarrow Ru(bpy)_3{}^{3+} \tag{1}$$

$$R_1R_2R_3N - e \rightarrow R_1R_2R_3N^{+\bullet} \rightarrow R_1R_2R_3N^{\bullet} + H^+ \tag{2}$$

$$R_1R_2R_3N^{\bullet} + Ru(bpy)_3{}^{2+} \rightarrow Ru(bpy)_3{}^{+} \tag{3}$$

$$R_1R_2R_3N^{\bullet} + Ru(bpy)_3{}^{3+} \rightarrow Ru(bpy)_3{}^{2+*} + \text{product1} \tag{4a}$$

$$Ru(bpy)_3{}^{+} + Ru(bpy)_3{}^{3+} \rightarrow Ru(bpy)_3{}^{2+} + Ru(bpy)_3{}^{2+*} \tag{4b}$$

$$Ru(bpy)_3{}^{2+*} \rightarrow Ru(bpy)_3{}^{2+} + h\nu \tag{5}$$

$$R_1R_2NR_3NR_1R_2 - 2e - H^+ \rightarrow R_1R_2N^{+\bullet}R_3N^{\bullet}R_1R_2 \tag{6}$$

$$R_1R_2N^{+\bullet}R_3N^{\bullet}R_1R_2 \rightarrow \text{product2} \tag{7}$$

3.2. ECL and electrochemistry at platinum electrodes

Fig. 4 shows the linear sweep voltammograms and ECL curves at the platinum electrode. N-butyldiethanolamine exhibits larger anodic current and slightly stronger ECL than 2-(dibutylamino)ethanol. It has been reported that the growth of oxide film at platinum electrodes dramatically inhibits the direct oxidation of tripropylamine [29], and the hydroxyethyl in 2-(dibutylamino)ethanol can promote the direct oxidation of amine group at platinum electrodes [39]. The faster oxidation of N-butyldiethanolamine over 2-(dibutylamino)ethanol demonstrates that two hydroxyethyl groups can also promote the oxidation of amines at platinum electrodes, and is more effective than one hydroxyethyl group.

Similar to the phenomena at the gold electrodes, both diamines HEEDA and HPEDA show significantly larger anodic currents and weaker ECL intensities than N-butyldiethanolamine and 2-(dibutylamino)ethanol at the platinum electrodes (Fig. 4). As mentioned above, the phenomena at the platinum electrodes may also be ascribed to more significant side reactions of diamines, such as the inner molecular side reaction between electrogenerated amine cation radicals and amine free radicals [34].

3.3. ECL and electrochemistry at glassy carbon electrodes

ECL intensity at the glassy carbon electrodes first increases with N-butyldiethanolamine concentrations up to 3 mM and then levels off as a result of side reactions at higher concentrations. In contrast to the phenomena at the gold and platinum electrodes, the ECL intensity of N-butyldiethanolamine at the glassy carbon electrodes is weaker than that of 2-(dibutylamino)ethanol despite that the oxidation of N-butyldiethanolamine is still faster (Fig. 5). The weaker ECL of N-butyldiethanolamine at the glassy carbon electrodes may be due to heavier side reactions [29,34].

HEEDA and HPEDA still show much weaker ECL than N-butyldiethanolamine and 2-(dibutylamino)ethanol at the glassy carbon electrodes. Contrary to the observation at the gold and platinum electrodes, HEEDA and HPEDA exhibit lower anodic currents than N-butyldiethanolamine and 2-(dibutylamino)ethanol at the glassy carbon electrodes. The different oxidation rates may result from the different interactions between these amines and different electrode materials.

3.4. Determination of $Ru(bpy)_3^{2+}$

The logarithmic plot of ECL versus the concentration of $Ru(bpy)_3^{2+}$ is linear in the range of 4.0×10^{-10} M–1.0×10^{-3} M (slope = 0.83934; intercept = 9.04845; correlation coefficient = 0.99325; $n = 8$) when the concentration of N-butyldiethanolamine is 15 mM and a gold electrode is used. The relative standard deviation is 2.7% for six determinations at a $Ru(bpy)_3^{2+}$ concentration of 1 μM, and the detection limit is 6.2×10^{-11} M.

4. Conclusions

ECL of monoamines and diamines containing different numbers of hydroxyl was investigated. N-butyldiethanolamine containing two hydroxyethyl is more effective than 2-(dibutylamino)ethanol containing one hydroxyethyl at the gold and platinum electrodes, and is the most effective coreactant reported so far. Both diamines HEEDA and HPEDA exhibit much weak ECL than the monoamines. The weaker ECL of diamines may result from more significant side reactions, such as the intramolecular side reaction between oxidative amine cation radicals and reductive amine free radicals. This study is helpful in search of new coreactants.

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