

Emission of Tris(2,2'-bipyridine)ruthenium(II) by Coreactant Electrogenerated Chemiluminescence: From O₂-Insensitive to Highly O₂-Sensitive

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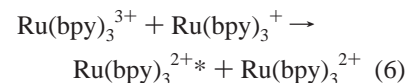
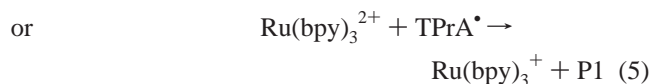
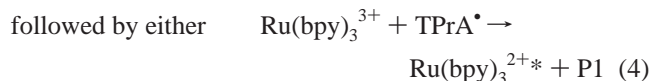
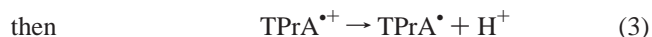
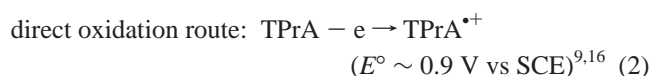
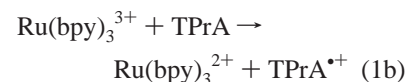
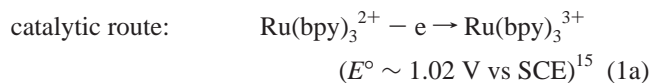
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We describe the influence of dissolved oxygen on the emission of Ru(bpy)₃²⁺ (bpy = 2,2'-bipyridine) by electrogenerated chemiluminescence (ECL) with tertiary amine as coreactant in aqueous solutions. The significance of the reactions between molecular oxygen and the ECL intermediate reducing radicals has been demonstrated for the first time. By varying the experimental conditions, the oxygen effect on different ECL routes of the Ru(bpy)₃²⁺/tri-*n*-propylamine (TPrA) system was examined. When coreactant direct oxidation played a predominant role in producing ECL, the maximum emission intensity, especially that of the low-oxidation-potential (LOP) ECL, could change from O₂-insensitive to highly O₂-sensitive with decreasing TPrA concentration. This behavior can be interpreted as follows: A large excess of intermediate reducing radicals was produced at high [TPrA], and the dissolved oxygen within the ECL reaction layer was completely reduced by these radicals and exerted no quenching effect on the emission. At low [TPrA], however, coreactant oxidation generated a relatively small amount of reducing intermediates, and molecular oxygen acted as an interceptor, destroying the intermediates before they participated in the ECL pathways, which led to the obvious reduction of the emission intensity. In the latter case, the less efficient LOP ECL route was more remarkably affected. When ECL was generated primarily via the catalytic route at high [Ru(bpy)₃²⁺], the reactions consuming the intermediate radicals by O₂ became insignificant, and the drop of emission intensity in the presence of oxygen could mainly be ascribed to the excited-state quenching. A similar oxygen effect was also observed for the Ru(bpy)₃²⁺/triethylamine (TEA) system.

Introduction

Electrogenerated chemiluminescence (ECL) experiments based on radical ion annihilation are usually conducted in aprotic solvents.¹ Water and oxygen are particularly harmful. However, ECL can also be observed in aqueous solutions via an alternative means of utilizing coreactants, such as organic acids and amines. The coreactant systems have made it possible to adopt the ECL technique in clinical analysis (immunoassay and DNA probes).² There has been little literature discussion regarding the oxygen effect on coreactant ECL. Previous studies on the Ru(bpy)₃²⁺ (bpy = 2,2'-bipyridine)/C₂O₄²⁻ system revealed that solution deaeration would increase the ECL efficiency by ~2-fold.^{3,4} This has been attributed to the quenching effect of oxygen on the Ru(bpy)₃²⁺ excited state. For another important and most commonly employed coreactant system, Ru(bpy)₃²⁺/tri-*n*-propylamine (TPrA), it is known that the oxygen influence is also not severe and a deaeration step is usually not required in the ECL measurement, although more light has been detected when this step was included.^{5–7} To achieve a better understanding of the oxygen effect, more detailed investigation is necessary.

It is generally accepted that coreactant ECL follows a mechanism involving the formation of a highly reducing intermediate species upon coreactant oxidation. The ECL mechanism of the Ru(bpy)₃²⁺/TPrA system has been of interest in many studies.^{5–14} On the basis of TPrA oxidation, two routes have been proposed:^{6–12}



where TPrA^{•+} = Pr₃N^{•+}, TPrA[•] = Pr₂NC[•]HCH₂CH₃, and P1 = Pr₂NC⁺HCH₂CH₃.

Previous studies suggested that the excited state was produced primarily by reaction 4, while reactions 5 and 6 were quite inefficient.^{7,9,17} The contributions of the catalytic route and the direct oxidation route to the overall ECL intensity depend on

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the relative concentrations of $\text{Ru}(\text{bpy})_3^{2+}$ and TPrA as well as the TPrA oxidation rate at the electrode.^{7–9,11}

Recently, a novel reaction pathway (eq 7), which involves the intermediacy of the TPrA cation radical, $\text{TPrA}^{\bullet+}$, was proposed.¹¹



Compared with the conventional ECL routes (catalytic and direct oxidation routes), a unique feature of the new emission pathway is that no oxidation of $\text{Ru}(\text{bpy})_3^{2+}$ is necessary to produce the excited state. Therefore, a low-oxidation-potential (LOP) ECL signal may be generated via the route (2)–(3)–(5)–(7) at a potential less positive than that required to form $\text{Ru}(\text{bpy})_3^{3+}$. Indeed, evident LOP ECL has been observed at a freshly polished glassy carbon (GC) electrode below 1.0 V versus SCE.^{8,11} Our recent study revealed that more intense LOP ECL could be achieved at a gold electrode in the presence of nonionic fluorosurfactant species in solution.¹⁴ It has been noted that facile TPrA direct oxidation is essential for the generation of the LOP ECL signal.

In this study, we examined the oxygen effects on different light emission routes of the $\text{Ru}(\text{bpy})_3^{2+}$ /TPrA system. The experimental results indicated that the reactions between the reducing ECL intermediates and dissolved molecular oxygen could significantly affect the emission when TPrA direct oxidation played an important role in producing the excited state. Interestingly, the ECL peak intensity, especially that of the LOP ECL signal, could change from O_2 -insensitive to highly O_2 -sensitive as the solution conditions were varied. Similar ECL behavior has also been observed for the $\text{Ru}(\text{bpy})_3^{2+}$ /triethylamine system.

Experimental Section

Chemicals. Tris(2,2'-bipyridyl)ruthenium(II) dichloride hexahydrate ($\text{Ru}(\text{bpy})_3\text{Cl}_2 \cdot 6\text{H}_2\text{O}$, min 98%), tri-*n*-propylamine (TPrA, 98%), triethylamine (TEA, 99.5%), and Zonyl FSO-100 [$\text{F}(\text{CF}_2\text{CF}_2)_{1-7}\text{CH}_2\text{CH}_2\text{O}(\text{CH}_2\text{CH}_2\text{O})_x\text{H}$] were purchased from Aldrich. Other chemicals were analytical reagent graded and used as received. All solutions were prepared with deionized water (Milli Q, Millipore). The pH of the phosphate buffer solution (PBS) containing TPrA or TEA was adjusted with concentrated NaOH or phosphoric acid.

Electrochemical and ECL Measurements. Cyclic voltammetry (CV) was performed with the model 600A electrochemical workstation (CH Instruments, Austin, TX). Glassy carbon (GC), platinum, and gold electrodes were polished with 0.05- μm alumina slurry to obtain a mirror surface and then were sonicated and thoroughly rinsed with Milli-Q water. The reference electrode was a saturated calomel electrode separated from the working cell by a salt bridge; all potentials reported in this paper are relative to the saturated calomel electrode (SCE). The counter electrode was a coiled Pt wire. When necessary, electrolyte solution was saturated with O_2 or N_2 via gas bubbling for at least 15 min, and a constant flow of O_2 or N_2 was maintained over the solution during the measurement. Before each experiment, the gold and platinum working electrodes were subjected to repeated scanning in the potential ranges from -0.5 to 1.4 or -0.65 to 1.2 V, respectively, in 0.15 M PBS until reproducible voltammograms were obtained. The modification of the gold electrode with FSO-100 was conducted by immersing the electrode in 0.5 wt % surfactant solution for 5 min. The ECL signal was measured with a photomultiplier tube (PMT, Hamamatsu R928) installed under

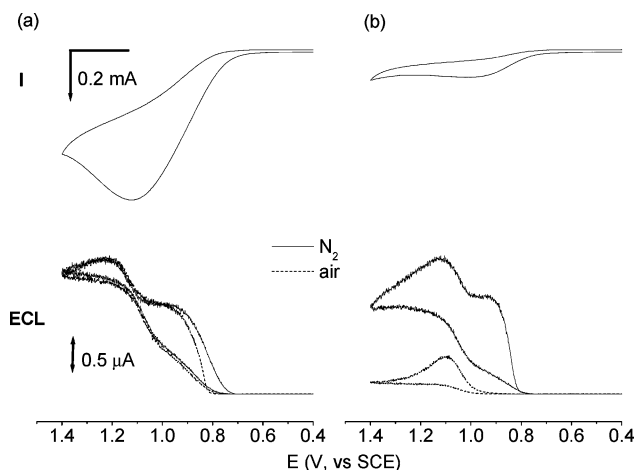


Figure 1. Cyclic voltammograms and their corresponding ECL curves of $1 \mu\text{M}$ $\text{Ru}(\text{bpy})_3^{2+}$ in the presence of 80 mM (a) or 7.5 mM (b) TPrA with 0.15 M PBS (pH 7.5) before and after deaeration. Electrode, 3-mm-diameter GC electrode. Scan rate, 0.1 V/s.

the electrochemical cell. A voltage of -800 V (or -600 V when 1 mM $\text{Ru}(\text{bpy})_3^{2+}$ was used) was supplied to the PMT with the Sciencetech PMH-02 instrument (Sciencetech Inc., Hamilton, Ontario, Canada). All experiments were performed at room temperature.

Results and Discussion

By changing the experimental conditions, the predominant ECL route of the $\text{Ru}(\text{bpy})_3^{2+}$ /TPrA system would vary,^{7–9,11} and the oxygen effects on different routes could be studied. First, a GC electrode was used to produce ECL in 0.15 M PBS (pH 7.5) containing $1 \mu\text{M}$ $\text{Ru}(\text{bpy})_3^{2+}$ and relatively concentrated TPrA. In this case, TPrA direct oxidation played an important role. Figure 1a shows that the oxidation of 80 mM TPrA displayed a broad irreversible anodic peak; meanwhile, two ECL waves were observed during the positive potential scan, similar to that reported previously.^{8,11} The main ECL peak appeared at ~ 1.2 V, which was related to the oxidation of $\text{Ru}(\text{bpy})_3^{2+}$; the other less intense ECL wave (i.e., LOP ECL) located at ~ 0.9 V. The maximum intensities of both ECL waves were almost uninfluenced by oxygen. However, when the TPrA concentration was reduced to 7.5 mM, the ECL signals became very sensitive to the presence of oxygen, as shown in Figure 1b. In air-saturated solution, only the main ECL peak appeared, while no LOP ECL wave was observed. After deaeration, the peak intensity of the main ECL signal increased by ~ 4 times, and an evident LOP ECL peak appeared. Note that the deaeration had no influence on the TPrA oxidation current. Figure 2 shows the intensities of the conventional and LOP ECL signals as a function of TPrA concentration. The oxygen affected the emissions significantly at low [TPrA].

A similar oxygen effect on the LOP ECL signal was also observed at an FSO-modified gold electrode. As demonstrated in our previous study,¹⁴ the adsorption of the nonionic fluorosurfactant molecules at the gold electrode would facilitate TPrA oxidation by rendering the electrode surface more hydrophobic and inhibiting the growth of the surface oxides in the potential region below 1.0 V. When the electrode potential was scanned to more positive values, the desorption of the surfactant species occurred, following which gold surface oxide formed rapidly and TPrA oxidation was suppressed. As a result, a sharp LOP ECL peak appeared in the potential range between 0.8 and 1.0 V. Figure 3 shows the dramatic change of the oxygen effect on the LOP ECL as the TPrA concentration was decreased from

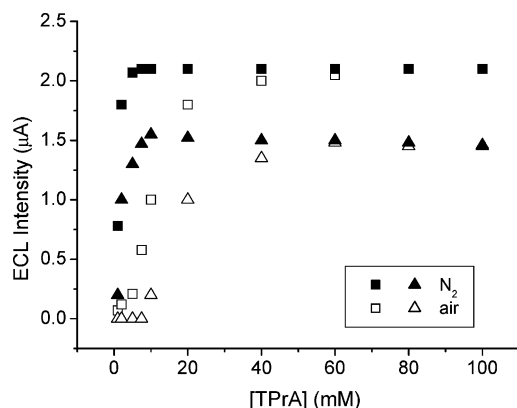
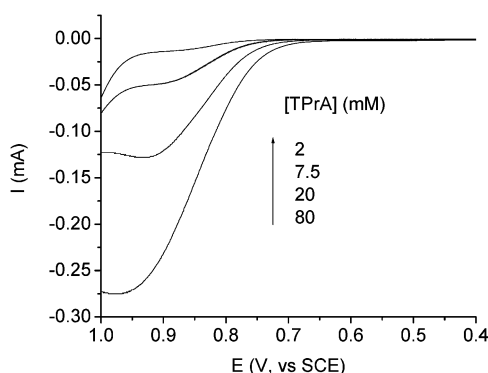


Figure 2. Intensities of the conventional ECL (squares) and LOP ECL (triangles) at the GC electrode (3-mm diameter) as a function of TPrA concentration. Electrolyte solution, 0.15 M PBS (pH 7.5) containing 1 μM $\text{Ru}(\text{bpy})_3^{2+}$. Scan rate, 0.1 V/s.

A. I - E Curves



B. ECL - E Curves

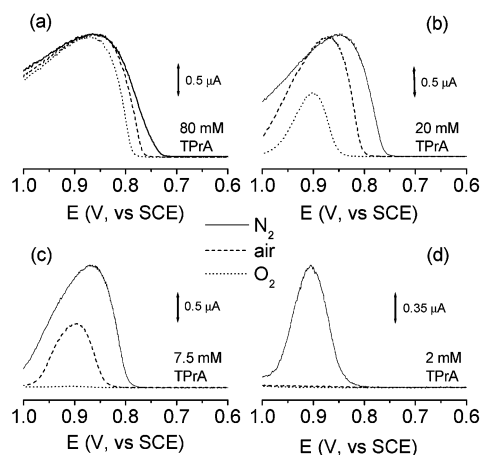


Figure 3. (A) Voltammograms and (B) low-oxidation-potential ECL curves of 1 μM $\text{Ru}(\text{bpy})_3^{2+}$ in the presence of various amounts of TPrA with 0.15 M PBS (pH 7.5) saturated by N_2 , air, or O_2 . Electrode, 2-mm-diameter gold electrode modified with FSO (see text). Scan rate, 0.1 V/s.

80 to 2 mM. The LOP ECL peak intensity was O_2 -insensitive at high [TPrA], but it became highly O_2 -sensitive at low [TPrA]. The LOP ECL intensity as a function of TPrA concentration is shown in Figure 4.

The high O_2 sensitivity of the ECL signals in dilute TPrA solution indicates that molecular oxygen could remarkably influence the emission process. It is known that molecular oxygen quenches the $\text{Ru}(\text{bpy})_3^{2+}$ excited state with a Stern–Volmer constant (K_{SV}) of $\sim 2 \times 10^3 \text{ M}^{-1}$ at 25 $^\circ\text{C}$.¹⁸ In O_2 -saturated aqueous solution ($[\text{O}_2] \sim 1.3 \times 10^{-3} \text{ M}$), the excited-

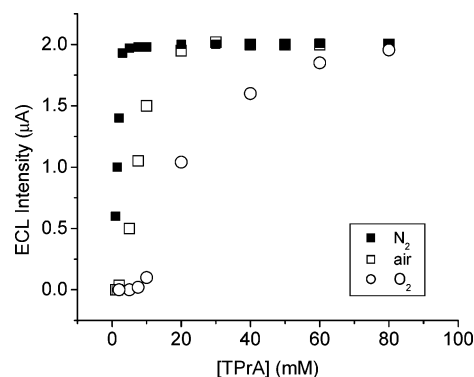
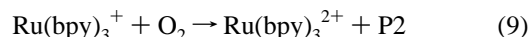


Figure 4. Intensity of the LOP ECL peak at the FSO-modified gold electrode (2-mm diameter) as a function of TPrA concentration. Electrolyte solution, 0.15 M PBS (pH 7.5) containing 1 μM $\text{Ru}(\text{bpy})_3^{2+}$. Scan rate, 0.1 V/s.

state quenching may not lead to a drop of emission intensity greater than 4-fold. Apparently, the oxygen quenching effect on the $\text{Ru}(\text{bpy})_3^{2+}$ excited state could not solely account for the O_2 sensitivity observed in the above experiments. It is probable that the side reactions between O_2 and the ECL reducing intermediate radicals, TPrA^{\bullet} (eq 8) as well as $\text{Ru}(\text{bpy})_3^{3+}$ (eq 9), played an important role. The oxidation potentials of TPrA^{\bullet} and $\text{Ru}(\text{bpy})_3^{3+}$ have been reported to be ~ -1.7 ¹⁶ and -1.52 V versus SCE,¹⁵ respectively; both species are strong reductants for molecular oxygen.



where P2 represents the products of O_2 reduction. Reactions 8 and 9 could compete with the ECL pathways by consuming the intermediate species, leading to the reduction of emission intensity. A similar quenching effect of nitrobenzenes on the ECL of the $\text{Ru}(\text{bpy})_3^{2+}$ /TPrA system has been reported recently.⁷ These compounds are also able to intercept the TPrA free radical before it reacts with $\text{Ru}(\text{bpy})_3^{3+}$.

Figure 1b shows obvious oxygen effects on both the conventional ECL (> 1.0 V) and the LOP ECL. Apparently, the LOP ECL signal was much more remarkably influenced. Note that, in air-saturated solutions, the LOP ECL was completely quenched as $[\text{TPrA}] < \sim 7.5$ mM at the GC electrode, while the conventional ECL signal was still evident even at $[\text{TPrA}] \sim 1$ mM (see Figure 2). Besides the excited-state quenching, molecular oxygen would affect the conventional ECL by intercepting TPrA^{\bullet} (eq 8) before it participated in reaction 4. Similarly, in the LOP ECL process, two crucial steps, eqs 5 and 7, would have to compete with reactions 8 and 9, respectively. Previous studies suggested that reaction 5 could be less efficient as compared with reaction 4.^{7,9} As $E_{\text{Ru}(\text{bpy})_3^{3+}/\text{Ru}(\text{bpy})_3^{2+}}^{\circ} = -1.08$ V versus SCE,¹⁵ the driving force of reaction 4 ($\Delta G_4^{\circ} \sim -0.62$ eV) is more favorable than that of reaction 5 ($\Delta G_5^{\circ} \sim -0.18$ eV). In a most recent report,¹⁷ the rate constant of reaction 7 (k_7) was estimated to be $\sim 10^6 \text{ M}^{-1} \text{ s}^{-1}$, much smaller than that of reaction 4 ($k_4 \sim 10^{10} \text{ M}^{-1} \text{ s}^{-1}$). Therefore, compared with step 4 in the conventional ECL route, both steps 5 and 7 in the LOP ECL route should be less favorable in competition with the side reactions consuming the intermediate radicals. As a result, the oxygen effect on the LOP ECL was more significant than that on the conventional ECL.

As the TPrA concentration was increased, more and more reducing species were produced following TPrA oxidation, and

their reactions with O_2 would exert less of an effect on the emission intensity. It is interesting to note that the maximum intensities of the ECL waves observed in Figures 1a and 3B(a) were almost uninfluenced by O_2 . Examining in detail the LOP ECL signals revealed that the emission started to occur at a more positive potential in the presence of O_2 . This indicates that most of the reducing intermediate species were destroyed by the side reactions in the initial stage of TPrA oxidation, and the LOP ECL was greatly diminished. Only when the TPrA oxidation current was large enough, the oxygen effect became ignorable. In these cases, the dissolved O_2 in the ECL reaction layer near the electrode surface might be completely reduced by the large excess reducing radicals; therefore, no oxygen effect, including $Ru(bpy)_3^{2+*}$ quenching, could be observed anymore.

The fact that rapid TPrA oxidation produced excess intermediate radicals relative to the dilute $Ru(bpy)_3^{2+}$ is also suggested by the ECL–[TPrA] profiles shown in Figures 2 and 4. In deaerated solutions, the intensity of the conventional ECL signal at the GC electrode increased quickly as [TPrA] increased and reached a plateau at [TPrA] > 7.5 mM (see Figure 2). The maximum emission intensity should be limited by the quantity of $Ru(bpy)_3^{2+}$. In air-saturated solutions, the increase of the ECL intensity became slower, and >40 mM TPrA was required to achieve the emission maximum. This can be ascribed to the consumption of the reducing intermediates by molecular oxygen. A similar relationship between LOP ECL intensity and TPrA concentration was also observed at the GC electrode as well as the FSO-modified gold electrode. Because of the more facile TPrA oxidation in the latter case, [TPrA] required to achieve the maximum LOP emission was reduced to 20 mM in air-saturated solutions. All of these experimental results indicate that a large excess of reducing radicals can be generated upon the oxidation of concentrated TPrA in the detection of trace $Ru(bpy)_3^{2+}$, and dissolved molecular oxygen will exert no quenching effect as long as the TPrA concentration is high enough and direct TPrA oxidation is fast. This demonstrated a unique advantage of the coreactant ECL technique in eliminating the influence of oxygen on emission.

It should be noted that a high concentration of TPrA does not necessarily guarantee the generation of excess intermediate radicals. For example, in a previous study using a gold working electrode,⁶ the ECL intensity rose with the increase of TPrA concentration up to 300 mM. This could result from the low TPrA oxidation rate at the bare gold electrode; in this case, the oxygen quenching effect could not be eliminated even at high [TPrA]. Our experiments showed that solution deaeration increased the ECL intensity by ~4-fold at a bare electrode as [TPrA] = 100 mM.

Next, we examined the oxygen effect on the ECL via the catalytic route. The ECL signal was produced by using a Pt electrode in 0.15 M PBS (pH 7.5) containing 1 mM $Ru(bpy)_3^{2+}$ and 1 mM TPrA. The surface oxides formed on the Pt electrode inhibited TPrA oxidation, minimizing the contribution of the direct oxidation route in the light emission.⁸ Similar to that reported for the $Ru(bpy)_3^{2+}/C_2O_4^{2-}$ system,^{3,4} solution deaeration increased the ECL peak intensity by ~2-fold (see Supporting Information Figure S1). As the TPrA concentration was changed in the range from 1 μ M to 1 mM, the oxygen effect remained the same. This could mainly be attributed to $Ru(bpy)_3^{2+*}$ quenching. Although the interception of the ECL intermediate radicals by molecular oxygen might not be completely ruled out, it should play a minor role; otherwise, the emission intensity should have been much greatly reduced

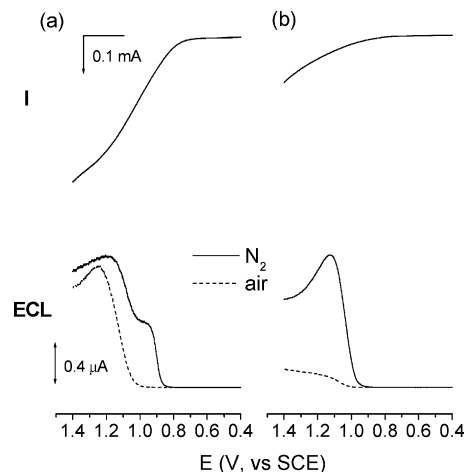


Figure 5. Voltammograms and their corresponding ECL curves of 1 μ M $Ru(bpy)_3^{2+}$ in the presence of 100 mM (a) or 20 mM (b) TEA with 0.15 M PBS (pH 7.5) before and after deaeration. Electrode, 3-mm-diameter GC electrode. Scan rate, 0.1 V/s.

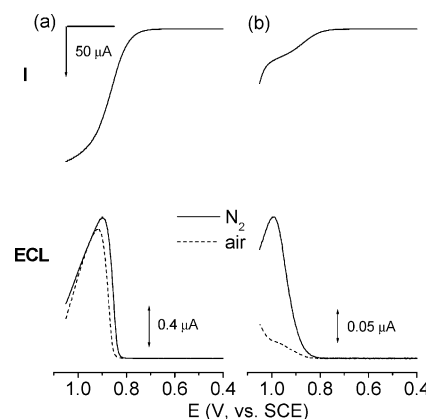


Figure 6. Low-oxidation-potential ECL curves in the presence of 100 mM (a) or 20 mM (b) TEA with 0.15 M PBS (pH 7.5) before and after deaeration. Electrode, 2-mm-diameter gold electrode modified with FSO (see text). Scan rate, 0.1 V/s.

due to the low concentration of TPrA used in these cases. In the catalytic route, a large amount of electrogenerated $Ru(bpy)_3^{3+}$ reacted rapidly with TPrA $^{\bullet}$ (eq 4). The rate constant of reaction 4 has been estimated to be as high as $\sim 10^{10} \text{ M}^{-1} \text{ s}^{-1}$.¹⁷ The reaction rate constant of TPrA $^{\bullet}$ with molecular oxygen (k_8) is unknown, but the above result suggested that k_8 should be $\ll 10^{10} \text{ M}^{-1} \text{ s}^{-1}$.

Tertiary amines other than TPrA may also be employed as coreactant for $Ru(bpy)_3^{2+}$ ECL. We examined the oxygen effect on the $Ru(bpy)_3^{2+}$ /triethylamine (TEA) system by using the same strategy. An ~2-fold enhancement of emission intensity was observed after solution deaeration for the ECL signal produced at the Pt electrode via the catalytic route (see Supporting Information Figure S2), suggesting the excited-state quenching by oxygen. Figure 5 shows the oxygen influences on the ECL signals at the GC electrode under the conditions of low $[Ru(bpy)_3^{2+}]$ and relatively high [TEA]. At [TEA] ~ 100 mM, the peak intensity of the conventional ECL wave was O_2 -insensitive, and an evident LOP ECL signal was observed in the deaerated solution.¹⁹ However, since the TEA oxidation rate was lower than the TPrA one, especially in the potential region below 1.0 V, the LOP ECL was completely quenched in the air-saturated solution. As [TEA] decreased to 20 mM, no LOP ECL appeared, and the conventional ECL signal became very sensitive to the presence of oxygen. At the FSO-modified gold electrode, more facile oxidation of TEA could be achieved and

more intense LOP ECL signals were obtained, as shown in Figure 6. The maximum intensity of the LOP ECL changed from O₂-insensitive at high [TEA] to highly O₂-sensitive at low [TEA], just like the behavior observed in the Ru(bpy)₃²⁺/TPrA system. Note that the LOP ECL signal at 20 mM TEA (Figure 6b) was much more O₂-sensitive than that at 20 mM TPrA (Figure 3B(b)) due to the lower oxidation rate of TEA. The oxidation of TEA produced cation and free radicals, (CH₃-CH₂)₃N^{•+} and (CH₃CH₂)₂NC[•]HCH₃. The TEA free radical is also a highly reducing species, and its oxidation potential has been estimated to be ~ -1.12 or -1.7 V versus SCE in acetonitrile or H₂O/acetonitrile mixed solutions.^{20,21} Dissolved oxygen could affect the ECL process following a similar mechanism as that described above.

Conclusions

Dissolved oxygen exerts various influences on coreactant ECL. Besides excited-state quenching, the reactions between molecular oxygen and the intermediate reducing radicals could significantly affect the ECL of Ru(bpy)₃²⁺ with TPrA as coreactant. When TPrA direct oxidation played a predominant role in producing ECL, the oxygen effect varied with the solution conditions. At high [TPrA], a large excess of reducing intermediates could be generated at GC or FSO-modified gold electrodes upon TPrA oxidation, by which the oxygen molecules within the ECL reaction layer might be completely reduced; as a result, the ECL peak intensities (both the conventional ECL and the LOP ECL) were insensitive to dissolved oxygen. In this case, the oxygen influence on ECL analysis was eliminated. On the other hand, the ECL signals, especially the LOP ECL, became highly O₂-sensitive at relatively low [TPrA]. The competition between the ECL steps and the side reactions involving oxygen remarkably reduced the emission intensity. On the basis of this behavior, it is possible to develop an optical method for dissolved oxygen sensing in solution. A high sensitivity may be achieved for different concentration ranges of O₂ by varying the quantity of TPrA. Further study in this direction is currently underway.

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Supporting Information Available: Figures showing ECL-potential curves. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References and Notes

- (1) For a review of ECL, see: (a) Faulkner, L. R.; Bard, A. J. In *Electroanalytical Chemistry*; Bard, A. J., Ed.; Marcel Dekker: New York, 1977; Vol. 10, p 1. (b) Knight, A. W.; Greenway, G. M. *Analyst* **1994**, *119*, 879. (c) Bard, A. J.; Debad, J. D.; Leland, J. K.; Sigal, G. B.; Wilbur, J. L.; Wohlstadter, J. N. *Chemiluminescence*. In *Encyclopedia of Analytical Chemistry: Applications, Theory and Instrumentation*; Meyers, R. A., Ed.; John Wiley & Sons: New York, 2000; Vol. 11, p 9842 and references therein. (d) Richter, M. M. *Chem. Rev.* **2004**, *104*, 3003. (e) *Electrogen-erated chemiluminescence*; Bard, A. J., Ed.; Marcel Dekker: New York, 2004.
- (2) (a) Blackburn, G. F.; Shah, H. P.; Kenten, J. H.; Leland, J.; Kamin, R. A.; Link, J.; Peterman, J.; Powell, M. J.; Shah, A.; Talley, D. B.; Tyagi, S. K.; Wilkins, E.; Wu, T. G.; Massey, R. J. *Clin. Chem.* **1991**, *37*, 1534. (b) Kijek, T. M.; Rossi, C. A.; Moss, D.; Parker, R. W.; Henchal, E. A. *J. Immunol. Methods* **2000**, *236*, 9. (c) Xu, X. H.; Bard, A. J. *J. Am. Chem. Soc.* **1995**, *117*, 2627. (d) Yu, H. J. *Immunol. Methods* **1998**, *218*, 1. (e) Hsueh, Y. T.; Smith, R. L.; Northrup, M. A. *Sens. Actuators* **1996**, *33*, 110. (f) Boom, R.; Sol, C.; Weel, J.; Gerrits, Y.; de Boer, M.; Wertheim-van Dillen, P. *J. Clin. Microbiol.* **1999**, *37*, 1489.
- (3) Rubinstein, I.; Bard, A. J. *J. Am. Chem. Soc.* **1981**, *103*, 512.
- (4) Malins, C.; Vandeloise, R.; Walton, D.; Vander Donckt, E. *J. Phys. Chem. A* **1997**, *101*, 5063.
- (5) Noffsinger, J. B.; Danielson, N. D. *Anal. Chem.* **1987**, *59*, 865.
- (6) Leland, J. K.; Powell, M. J. *J. Electrochem. Soc.* **1990**, *137*, 3127.
- (7) Gross, E. M.; Pastore, P.; Wightman, R. M. *J. Phys. Chem. B* **2001**, *105*, 8732.
- (8) Zu, Y.; Bard, A. J. *Anal. Chem.* **2000**, *72*, 3223.
- (9) Kanoufi, F.; Zu, Y.; Bard, A. J. *J. Phys. Chem. B* **2001**, *105*, 210.
- (10) Zu, Y.; Bard, A. J. *Anal. Chem.* **2001**, *73*, 3960.
- (11) Miao, W.; Choi, J.-P.; Bard, A. J. *J. Am. Chem. Soc.* **2002**, *124*, 14478.
- (12) Honda, K.; Yoshimura, M.; Rao, T. N.; Fujishima, A. *J. Phys. Chem. B* **2003**, *107*, 1653.
- (13) Zhou, M.; Heinze, J.; Borgwarth, K.; Grover, C. P. *ChemPhysChem* **2003**, *4*, 1241.
- (14) Li, F.; Zu, Y. *Anal. Chem.* **2004**, *76*, 1768.
- (15) (a) Lin, C. T.; Boettcher, W.; Chou, M.; Creutz, C.; Sutin, N. *J. Am. Chem. Soc.* **1976**, *98*, 6536. (b) Sutin, N.; Creutz, C. *Adv. Chem. Ser.* **1978**, *168*, 1. (c) Sutin, N. *Acc. Chem. Res.* **1982**, *15*, 273. (d) Sutin, N. *Prog. Inorg. Chem.* **1983**, *30*, 441. (e) Krishnan, C. V.; Creutz, C.; Schwarz, H. A.; Sutin, N. *Inorg. Chem.* **1983**, *22*, 5617.
- (16) Lai, R. Y.; Bard, A. J. *J. Phys. Chem. A* **2003**, *107*, 3335.
- (17) Wightman, R. M.; Forry, S. P.; Maus, R.; Badocco, D.; Pastore, P. *J. Phys. Chem. B* **2004**, *108*, 19119.
- (18) (a) Lin, C.-T.; Sutin, N. *J. Phys. Chem.* **1976**, *80*, 97. (b) Winterle, J. S.; Klinger, D. S.; Hammond, G. S. *J. Am. Chem. Soc.* **1976**, *98*, 3719.
- (19) The low-oxidation-potential ECL of the Ru(bpy)₃²⁺/TEA system should follow a mechanism similar to that proposed for the Ru(bpy)₃²⁺/TPrA system (see ref 11).
- (20) Wayner, D. D. M.; McPhee, D. J.; Griller, D. *J. Am. Chem. Soc.* **1988**, *110*, 132.
- (21) Bhattacharyya, K.; Das, P. K. *J. Phys. Chem.* **1986**, *90*, 3987.