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Enhanced Electrogenerated Chemiluminescence of Tris (2,2′-bipyridyl) Ruthenium (II)–$S_2O_8^{2-}$ System by Sodium Dodecyl Sulfate

Sung Chul Kang, Sooil Oh, and Kang-Jin Kim*

Department of Chemistry, Korea University, Seoul 136-701. *Received April 25, 1990*

The electrochemical reduction and electrogenerated chemiluminescence (ECL) of Ru(bpy)$_3^{2+}$–$S_2O_8^{2-}$ in CH_3CN–H_2O solution were studied in the presence of sodium dodecyl sulfate (SDS) as an anionic surfactant. SDS enhanced the ECL and the fluorescence intensities and lengthened the duration of ECL due to the solubilization of reactants and possibly to the stabilization of ECL intermediates in the SDS micellar environment.

Introduction

Interests in the electrogenerated chemiluminescence (ECL) of tris(2,2′-bipyridyl) ruthenium(II)(Ru(bpy)$_3^{2+}$) in aqueous or acetonitrile–water solutions have been increasing rapidly since Ru(bpy)$_3^{2+}$ as an ECL label can be used to determine low concentrations of biologically important compounds.[1,2] In particular, the ECL mechanism of Ru(bpy)$_3^{2+}$–$S_2O_8^{2-}$ system in acetonitrile–water solution estabilished recently by White and Bard are based on the following reaction sequence.[2]

$$\text{Ru(byp)}_3^{2+} + e^- \rightarrow \text{Ru(bby)}_3^{+} \tag{1}$$

$$\text{Ru(bby)}_3^{+} + S_2O_8^{2-} \rightarrow \text{Ru(bpy)}_3^{2+} + SO_4^{\cdot-} + SO_4^{2-} \tag{2}$$

$$\text{Ru(bpy)}_3^{+} + SO_4^{\cdot-} \rightarrow \text{Ru(bpy)}_3^{2+*} + SO_4^{2-} \tag{3}$$

Because Ru(bpy)$_3^{+}$ is unstable in aqueous solutions and $S_2O_8^{2-}$ has a low solubility in CH_3CN solutions[3], the CH_3CN–H_2O mixed solutions are used to produce intense ECL emission.

Electrogenerated reactive intermediates are often stabilized in micellar media on the reductive electrochemical system. For example, Saveant *et al.*[4] have reported a remarkable stabilization of the electrogenerated anion radical of phthalonitrile in the presence of cationic micelles and suggested that the observed 250 fold decrease in the rate of protonation of the anion radical was due to its association with the positively charged micelles. On the other hand, Blount *et al.*[5] found that anionic micelles in the presence of LiCl electrolyte were capable of stabilizing the nitrobenzen anion radical to the point where it become detectable by cyclic voltammetry at 50 mV/sec.

Recently, Ouyang and Bard[6] examined the oxidative electrochemistry and ECL of Os(bpy)$_3^{2+}$, and suggested that Os(bpy)$_3^{2+}$ interacted most strongly with anionic micelles, and both the electrochemical response and ECL in the presence of oxalate were suppressed. Bard and coworkers[7] had previously shown that the anionic micelles associated more strongly with methylviologen cationic radical($MV^{\cdot+}$) than the dication (MV^{2+}) form and Ru(bpy)$_3^{2+}$ is bound into Nafion more strongly than Ru(bpy)$_3^{3+}$. Their results were explained by the hydrophobic interactions between the given substrate and the micellar hydrocarbon core or the nonpolar regions of Nafion.

However, micellar systems have not been utilized for the ECL of Ru(bpy)$_3^{2+}$–$S_2O_8^{2-}$ system, although there are some reports concerning the CL improvements of lucigenin in micelle solutions.[8,9] Therefore, in this paper we attempt to describe the effect of sodium dodecyl sulfate (SDS) as an anionic surfactant for the purpose of enhancing the ECL efficiency of Ru(bpy)$_3^{2+}$–$S_2O_8^{2-}$ system. Possible causes of the enhancement are discussed.

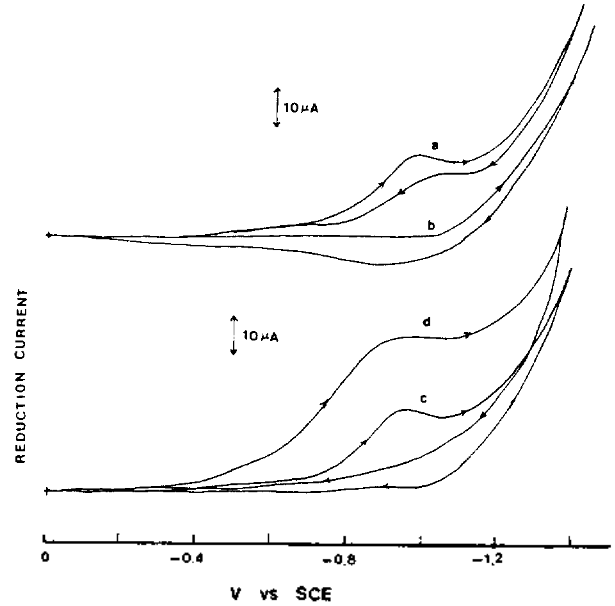

Figure 1. Cyclicvoltammograms at Pt electrode in CH_3CN-H_2O solutions containing (a) 7 mM $(NH_4)_2S_2O_8$, (b) 1 mM $Ru(bpy)_3Cl_2$, (c) 1 mM $Ru(bpy)_3Cl_2$ + 7 mM $(NH_4)_2S_2O_3$, (d) 1 mM $Ru(bpy)_3Cl_2$ + 7 mM $(NH_4)_2S_2O_8$ + 10 mM SDS. The supporting electrolyte was 0.1 M TMAP. The scan rate was 50 mV/sec.

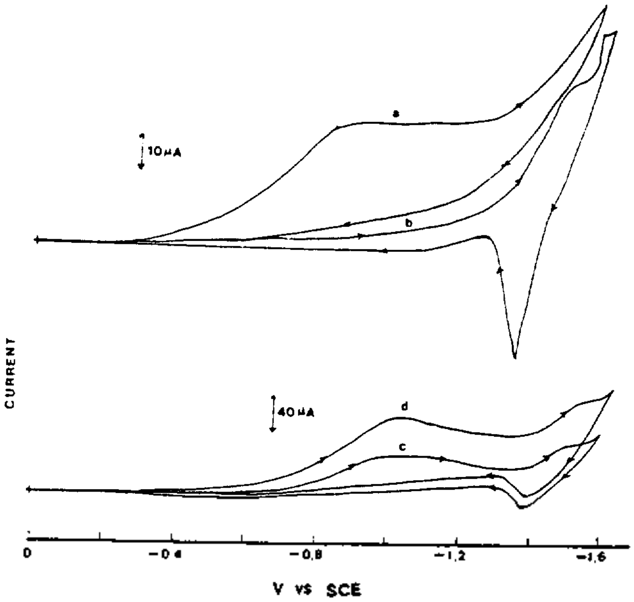

Figure 2. Cyclicvoltammograms at GC electrode in CH_3CN-H_2O solutions containing (a) 7 mM $(NH_4)_2S_2O_8$, (b) 1 mM $Ru(bpy)_3Cl_2$, (c) 1 mM $Ru(bpy)_3Cl_2$ + 7 mM $(NH_4)_2S_2O_8$, (d) 1 mM $Ru(bpy)_3Cl_2$ + 7 mM $(NH_4)_2S_2O_8$ + 10 mM SDS. The supporting electrolyte was 0.1 M TMAP. The scan rate was 50 mV/sec.

Experimental

$Ru(bpy)_3Cl_2$ was prepared by following the method of Bard *et al.*[1b] and identified with UV and fluorescence spectra. $(NH_4)_2S_2O_8$ (Junsei), tetramethylammonium perchlorate (TMAP) obtained from Fluka, acetonitrile (Junsei), sodium dodecyl sulfate (New Japan), cetyltrimethylammonium bromide (CTAB) (BDH), Triton X-100 (Shinyo) were used as received. All solution were deoxygenated with a stream of nitrogen gas within an electrochemical cell before measurements.

The electrochemical experiments were carried out in a three electrode cell with a working electrode, a Pt gauge counter electrode separated by a Vycor frit, and a SCE (Fisher Scientific) reference electrode connected to the analyzing compartment through a Vycor frit. The working electrode was either a Pt wire sealed into glass or a glassy carbon (GC) polished with raybrite A. All the potentialts given here are referred to the SCE. ECL and electrochemical measurements were performed with a Princeton Applied Research model 273 potentiostat/galvanostat and a RE0091 X-Y recorder ECL and fluorescence spectra were recorded on a Hitachi 650-60 spectrofluorimeter. Absorption spectra were obtained with a Cary 17D spectrophotometer.

Results and Discussion

Cyclic Voltammetry. Cyclic voltammetry was studied on the $Ru(bpy)_3^{2+}-S_2O_8^{2-}$ system in CH_3CN-H_2O (1:1 by volume) containing 0.1 M TMAP to observe the electrochemical changes upon the addition of surfactants. Mixed solvent was used here because the ECL was not observed in aqueous solutions.[3] Figure 1a and 2a present the cyclic voltammograms of 7 mM $(NH_4)_2S_2O_8$ at Pt and GC electrode, respectively. Scan rate was 50 mV/sec. Both figures show a broad cathodic wave corresponding to the irreversible one-electron reduction of $S_2O_8^{2-}$ at around −0.9V. The peak potential at Pt electrode is in disagreement with that of White and Bard[2] and the cause of the discrepancy is not clear.

Cyclic voltammograms of 1 mM $Ru(bpy)_3^{2+}$ in the mixed solutions at Pt and GC electrodes are shown in Figure 1b and 2b, respectively. At GC electrode a cathodic wave of a diffusional shape starts at about −1.4 V. At about −1.5 V, a relatively sharp current peak occurs, which may be attributed to strong adsorption of a uncharged complex.[2] At Pt electrode, however, neither the reduction of $Ru(bpy)_3^{2+}$ nor the sharp peak could be observed due to the overwhelming proton reduction and subsequent hydrogen evolution. When both 7 mM $S_2O_8^{2+}$ and 1 mM $Ru(bpy)_3^{2+}$ were present, the currents at potentials around −1.4 V were slightly increased and that at −1.5 V was decreased (Figure 2c).

The effect of micelle on the electrochemical behavior of $Ru(bpy)_3^{2+}-S_2O_8^{2-}$ system were investigated. In the presence of SDS above critical micelle concentration (cmc) the reduction potentials remained almost the same as can be seen in Figure 1d and 2d. The cmc of SDS in the system was estimated to be 3.0×10^{-3} M by florescence emission of $Ru(bpy)_3^{2+}$. However, the reduction currents were nearly double over the range of pctential investigated in comparison with those obtained without SDS. The increase in the reduction currents may be attributed to the enrichment of the reagents on the SDS micelles. Similar cyclic voltammetry with CTAB or Triton X-100 were performed and contrary to that with SDS, the resuls showed decrease in the reduction currents at all potentials.

Enhancing of ECL by SDS. ECL from Pt or GC electrode surface was studied in CH_3CN-H_2O solutions contain-

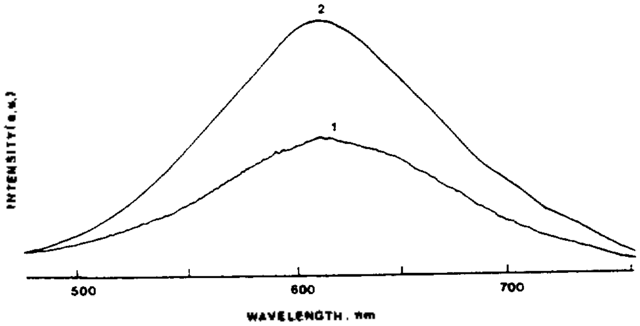

Figure 3. Comparison of ECL spectrum (1) with fluorescence emission spectrum (2) at Pt electrode in CH_3CN-H_2O solutions containing Ru(bpy)$_3^{2+}$ and $S_2O_8^{2-}$.

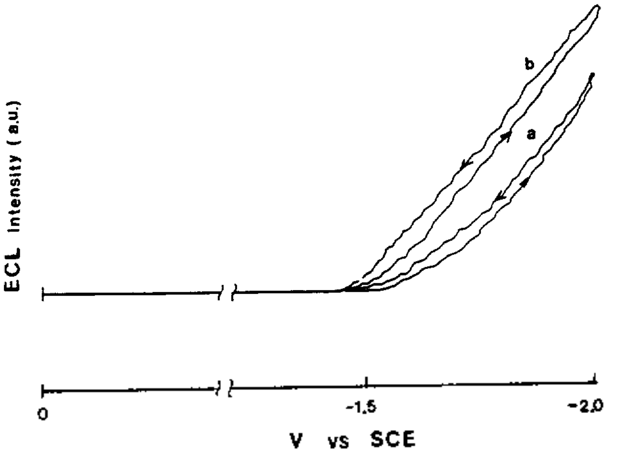

Figure 4. Effect of applied potential on the ECL intesities of solutions containing (a) Ru(bpy)$_3^{2+}$ + $S_2O_8^{2-}$, (b) Ru(bpy)$_3^{2+}$ + $S_2O_8^{2-}$ + SDS at Pt electrode. The scan rate was 50 mV/sec.

ing both Ru(bpy)$_3^{2+}$ and $S_2O_8^{2-}$. In the absence of either Ru(bpy)$_3^{2+}$ or $S_2O_8^{2-}$ ECL was not observed. Figure 3 shows a typical intensity-potential curve of Ru(bpy)$_3^{2+}$-$S_2O_8^{2-}$ at Pt electrode in CH_3CN-H_2O solutions. The noise in the figure is due to H_2 evolution. The figure demonstrates that the direct electrogeneration of Ru(bpy)$_3^+$ is necessary to produce Ru(bpy)$_3^{2+*}$. In Figure 4, typical enhancement of ECL intensity by SDS is indicated. The observed ECL spectrum is similar to the fluorescence emission spectrum of Ru(bpy)$_3^{2+}$ obtained in the CH_3CN-H_2O solution with the same maximum wavelength at 611 nm, indicating that the excited singlet state of Ru(bpy)$_3^{2+}$ is responsible for both ECL and fluorescence emission. Similar ECL in the presence of CTAB and Triton X-100 were studied and the relative integrated ECL intensities, I_{ECL}, are compared in Table 1. The result shows decrase in I_{ECL} for both CTAB and Triton X-100. Thus their micellar effects were not pursued further. Table 2 lists I_{ECL} *vs.* the concentration of SDS and the part of the data are plotted in Figure 5. The ECL intensity increases morotonically as the [SDS] increases. An attempt has been made to interpret the enhancement in I_{ECL} *vs.* [SDS] as follows.

The ECL efficiency, ϕ_{cl}, may be expressed as the product of the overall excited state yield of Ru(bpy)$_3^{2+*}$, ϕ_{ex}, and that of the fluorescence quantum yield of Ru(bpy)$_3^{2+}$, ϕ_f where

Table 1. Relative Integrated ECL Intensities (I_{ECL}) of 1.0×10^{-5} M Ru(bpy)$_3^{2+}$-20 mM $S_2O_8^{2-}$ in the Presence of Surfactants. Supporting Electrolyte was 1.0 M TMAP

Surfactant	Concentration, M	I_{ECL}, arb. units
non	—	1.0
SDS	8.0×10^{-3}	6.2
CTAB	8.5×10^{-4}	0.3
Triton X-100	1.8×10^{-4}	0.4

Table 2. Effect of SDS Concentration on the Relative Integrated ECL in Tensities (I_{ECL}) and Duration Time (T_d) of ECL of 1.0×10^{-5} M Ru(byp)$_3^{2+}$ and 20 mM $S_2O_8^{2-}$ in CH_3CN-H_2O. Supporting Electrolyte was 1.0 M TAMP

[SDS], 10^{-3} M	I_{ECL}, arb. units	T_d, min
0	1.0	28
1	2.2	40
3	3.8	52
5	5.0	58
8	6.2	70
10	7.0	80
15	7.2	90

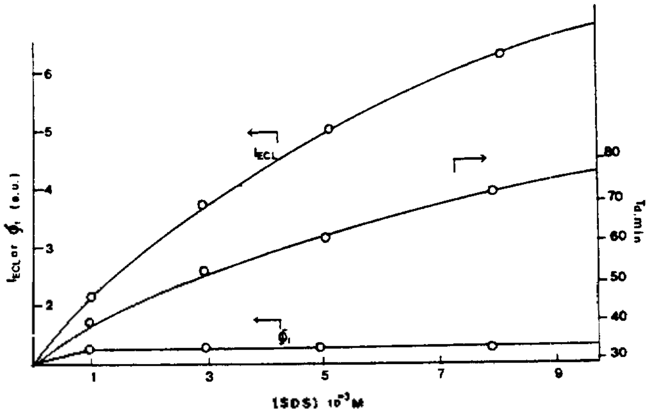

Figure 5. Effect of SDS contration of the ECL intensities. I_{ECL}, relative fluorescence quantum yield, ϕ_F, and duration time, T_d, of Ru(bpy)$_3^{2+}$-$S_2O_8^{2-}$ in CH_3CN-H_2O. Supporting electrolyte was 0.1 M TMAP.

$$\phi_{cl} = k\phi_{ex}\phi_f \qquad (4)$$

k is a propotionality constant. Since the observed shape of the fluorescence emission of Ru(bpy)$_3^{2+}$ *vs.* time in the presence of SDS micelles was not varied and the concentration of Ru(bpy)$_3^{2+}$ was kept constant, the maximum fluorescence emission intensity, I_f, corrected for absorbance at the excitation wavelength, A, should be proportional to ϕ_f

$$I_f / A \propto \phi_f \qquad (5)$$

The calculated values of I_f/A by Eq. (5) are presented in Figure 5. As can be estimated from Figure 5, SDS micelles apparently lead ϕ_f to increase by approximately 20%.

Since the reduction currents were increased nearly by a factor of two in the presence of SDS (Figure 1d), the yield of $Ru(bpy)_3^+$ may be assumed to be doubled, followed by doubling the yield of SO_4^-. Therefore ϕ_{ex} from Eq. (3) may be assumed to gain four fold increase at most in the SDS micellar environment.

Now, taking into account the 20% increase in ϕ_f, one may expect ϕ_{cl} to be increased at most by a factor of 4.8 from Eq. (4). However, as shown in Figure 5 experimental data suggest that the relative ECL intensity exceeds the expected increase above 5.0×10^{-3} M SDS. That is, the increases in ϕ_{ex} and ϕ_f cannot account for the large increase in ECL intensity *vs.* [SDS] beyond 5.0×10^{-3} M SDS. The cause of the additional increase in ϕ_{cl} is speculated due to the stabilization of the reduction intermediate $Ru(bpy)_3{}^+$ and/or SO_4^-. The explanation may be supported by a recent report, where the electrochemically produced anion radical of nitrobenzen was greatly stabilized for a relatively strong interaction between the radical and the SDS micellar environment.[5] In addition $Ru(bpy)_3{}^+$ can associate more strongly with SDS micellar core than $Ru(bpy)_3{}^{2+}$ by hydrophobic interaction.[7] Furthermore, the duration time, defined as the time required for ECL intensity to decay to zero, was lengthened in the presence of SDS micelles as can be seen from Figure 5 and Table 2, which possibly support the stabilization of the reaction intermediate.

Lastly, it was considered to be worth mentioning the possibility of adsorption of SDS on Pt electrode. Bard *et al.*[1b] have reported that the oxidation potential of $Ru(bpy)_3{}^{2+}$ in the presence of liver tissue shifts to a higher potential due to possible adsorption of the biological compound, thus changing the surface properties of the electrode. The shift of oxidation potential occurs also $Os(bpy)_3{}^{2+}$ interacts with SDS micelles by a strong hydrophobic interaction.[6]

In the present study, however, no such potential shift was observed for the reduction of $Ru(bpy)_3{}^{2+}$ in the presence of SDS at Pt electrode (Figure 1d). Thus it appears unlikely to assume that SDS micelles were adsorbed on Pt electrode. For negatively charged SDS micelles were presmably repelled from the cathode. Furthermore, since hydrogen was being evolved at Pt electrode over the pontential region negative of -1.4 V, SDS was apparently prevented from adsorbing to the electrode surface.

Therefore the ECL enhancement in this study appears not to be attributable to the adsorption of SDS on Pt electrode. Rather, it could be due to the solubilization of reactants and possible stabilization of ECL intermediates in the SDS micellar environment.

Acknowledgement. We are grateful for the financial support for this work from the Korea Science and Engineering Foundaiton.

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A Study of Phase Transition of Lipids and Lipid Mixtures by Differential Scanning Calorimetry

Nam Hong Kim, Sung Bae Roh, and Won Woo Park*

College of Pharmacy, Pusan National University, Pusan 609-390
**Department of Industrial Chemistry, Pusan Junior College, Pusan 616-092*

Received May 10, 1990

The simple binary mixtures of pure phospholipids such as DPPC:DSPC and DPPC:DPPA were investigated with differential scanning calorimeter. The thermotropic properties of DPPC and DSPC mixtures did not deviate much from the ideal curves that was obtained on varying either the temperature or the relative proportions of phospholipids. This means that the two phospholipids are completely miscible in the liquid and solid phase. But the thermotropic properties of DPPC and DPPA mixture deviated much from the ideal phase diagram. It resulted from the repulsion between the head groups of acidic phospholipids. Heat capacity suggested by Harden McConell was calculated. The larger C_p was, the sharper the endothermic peak became.