Surface Accumulable Coreactant for Bright Electrogenerated Chemiluminescence at Trace Level Concentrations

Ryoji Kurita,*[1] Kohei Nakamoto,[1,2] Dai Kato,[1] and Osamu Niwa*[1,2]
[1]*National Institute of Advanced Industrial Science and Technology (AIST),*
Tsukuba Central 6, 1-1-1 Higashi, Tsukuba 305-8566
[2]*Graduate School of Pure and Applied Science, University of Tsukuba,*
1-1-1 Tennodai, Tsukuba 305-8573

(Received April 16, 2009; CL-090377; E-mail: r.kurita@aist.go.jp, niwa.o@aist.go.jp)

We applied trimethylammonioethanethiol as an electrogenerated chemiluminescence coreactant for obtaining bright emission in a low concentration region. This is because trimethylammoniomethanethiol has the bifunctional properties of luminescence acceleration and surface accumulation on a gold electrode surface.

Electrogenerated chemiluminescence (ECL) has attracted great interest over the past two decades in relation to converting electrical energy into radiative energy,[1] essentially due to its very high potential for a wide range of applications including biological analysis, light sources, and displays. The most important and frequently used luminophore in ECL research is tris(2,2′-bipyridyl)ruthenium(II) ($Ru(bpy)_3^{2+}$) because of its strong luminescence and good solubility in a variety of solvents.[2] Furthermore, strong luminescence is observed when $Ru(bpy)_3^{2+}$ and tripropylamine as a coreactant are simultaneously oxidized on an electrode under physiological conditions.[3] The mechanism of this reaction is very complicated in that the oxidation product of the tripropylamine radical transfers an electron to the bipyridine of $Ru(bpy)_3^{+/3+}$. This provides conspicuously bright ECL in the $Ru(bpy)_3^{2+}$ system, and a commercially available ECL analyzer uses this $Ru(bpy)_3^{2+}$ and coreactant system.[4] Conventional bioassay systems using absorbance or fluorescence detection have been replaced with luminescence-based detection systems including ECL. This is because an ECL-based analyzer can achieve a high signal-to-noise ratio easily and inexpensively since no excitation light is needed. Unlike conventional optical detection techniques, ECL emission occurs very close to the electrode surface. Therefore, it is very important to increase the active species concentration on or near the electrode to enhance the sensitivity.[5]

Here, we present the first report of sulfanylalkylated quaternary ammoniums (Figure 1), which are bifunctional molecules with both ECL acceleration and surface accumulation effects. We found that quaternary ammoniums, which are considered to be an ECL nonemission coreactant owing to the lack of lone pairs at their nitrogen atoms, can be used as a coreactant as a result of sulfanylalkylation. In addition, the effective surface accumulation also helps to increase the electrode surface concentration of sulfanylalkylated quaternary ammoniums because thiols form a monolayer on a gold surface.

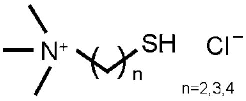

Figure 1. Sulfanylalkylated quaternary ammoniums as a coreactants for ECL. Trimethylammonioethanethiol (*n* = 2), -propanethiol (*n* = 3), and -butanethiol (*n* = 4). The details of synthesis are provided in the Supporting Information.[6]

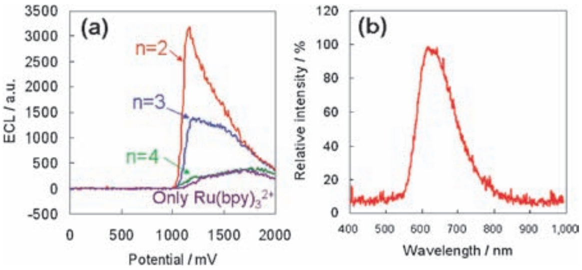

Figure 2. a) Variations in ECL intensity with increases in the electrode potential at a scan rate of $10\,mV\,s^{-1}$. b) ECL spectra of $Ru(bpy)_3^{2+}$ with trimethylammonioethanethiol.

Figure 2 shows the variations in ECL intensity observed when the potential of a gold electrode was scanned from 0 to 2000 mV in pH 7.0 phosphate buffer containing 2 mM $Ru(bpy)_3^{2+}$. The gold electrode was immersed in 1 mM of synthesized sulfanylalkylated quaternary ammoniums before the ECL measurements. There was a clear ECL increase on the electrode with a monolayer of trimethylammonioethanethiol (*n* = 2) and -propanethiol (*n* = 3). The ECL with trimethylammonioethanethiol was bright, and we were able to obtain the ECL spectrum using a CCD-based spectrometer as shown in the Figure 2. The spectrum had a 610-nm peak as found with $Ru(bpy)_3^{2+}$-based ECL. However, the ECL intensity was not enhanced when the electrode was modified with trimethylammoniobutanethiol (*n* = 4), whose intensity was the same as the background level (only $Ru(bpy)_3^{2+}$). This is because the deprotonation is induced when the alkyl chain is short by the synergetic effect of thiol and quaternary ammonium groups. Details are described with cyclic voltammetric results in Figure 3.

Figure 3a shows the variations in ECL intensity as the electrode potential increases on a gold surface with trimethylammonioethanethiol. The gold electrode was immersed in 10 nM–1 mM of trimethylammonioethanethiol for 30 min before the ECL measurements. The ECL intensity rapidly increased at 1150 mV and then gradually decreased to the background level. Figure 3b shows cyclic voltammograms obtained on the gold electrode with and without trimethylammonioethanethiol. The subtracted voltammogram shown in the inset in Figure 3b reveals two oxidation peaks. The peak at 900 mV is thiol oxidation since thiol–gold binding is oxidized to sulfonyl directly in a neutral solution at the potential.[7] We also observed a further ox-

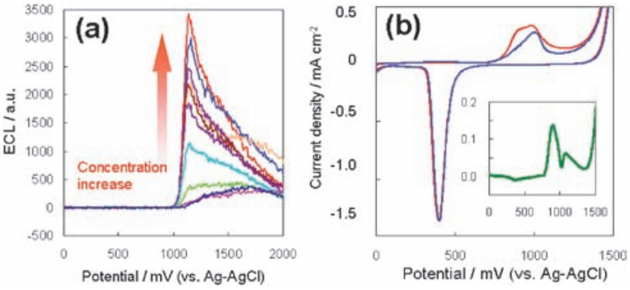

Figure 3. a) Variations in ECL emission on a gold electrode that acquires an accumulated monolayer of trimethylammonioethanethiol as the potential increases. b) Cyclic voltammograms for a gold electrode preconcentrated with (red) and without (blue) trimethylammonioethanethiol. The inset shows the subtracted voltammograms obtained by deducting the current shown in blue from that shown in red.

idation peak at 1150 mV, which has the same potential as the ECL emission peak in Figure 3a.

Primary, secondary, and tertiary amine based ECL has been reported as a result of the formation of a strong reducing radical by the oxidation of lone pairs at a nitrogen atom, followed by deprotonation at a neighboring carbon.[8] Therefore there has been no report describing ECL using quaternary ammoniums, which have no lone pairs. Our result is the first report of ECL emission achieved using quaternary ammoniums. Although we are currently investigating the mechanism in detail, we consider the oxidation peak at 1150 mV to indicate the deprotonation of an alkyl chain as in the previously described amine-based coreactant ECL system.[8] This is because the ECL emission at 1150 mV increased with increasing pH (data not shown), and H^+ at carbon adjacent to sulfonyl and quaternary ammonium groups can be easily removed as a result of electron-withdrawing properties. Furthermore, the synergetic effect of two electron-withdrawing groups would be required because we observed no ECL on either the gold or carbon electrode when we used trimethylammonioethanol [$(CH_3)_3N^+(CH_2)_2OH$] and ethanethiol (CH_3CH_2SH) as coreactants. In addition, the ECL emission was very weak when the alkyl chain was long ($n = 4$) as shown in Figure 2a.

Figure 4 compares the ECL intensity of trimethylammonioethanethiol with that of tripropylamine, which provides the brightest emission yet reported. The ECL with tripropylamine was stronger than that with trimethylammonioethanethiol in the high concentration range because tripropylamine exhibits higher ECL efficiency, and the coreactant concentration is sufficiently high for us to disregard the surface accumulation effect of trimethylammonioethanethiol. However, we observed stronger ECL with trimethylammonioethanethiol at a low concentration of less than 5 µM with 30-min preconcentration despite washing the trimethylammonioethanethiol in a bulk solution. This is because the ECL with tripropylamine was almost linear with the tripropylamine concentration, whereas the ECL with our material was maintained by the preconcentration effect on a gold surface by the gold–thiol bonding. For example, the ECL intensity with 0.2 µM trimethylammonioethanethiol is 10 times greater than that of tripropylamine.

In conclusion, we found that trimethylammonioethanethiol works as a luminophore coreactant, and the strong emission appears in the low concentration region. We have previously reported highly sensitive immunoassays realized by measuring trimethylammonioethanethiol formed by an antibody labeled enzymatic reaction detected with cathodic stripping or detected from surface plasmon resonance after accumulation on a gold surface.[9] Since we also confirmed that it was possible to tune the ECL sensitivity range by changing the accumulation time, the materials and results presented here will be promising an ECL based bioaffinity assay with an extreme low detection limit.

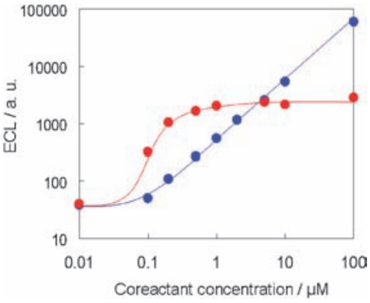

Figure 4. Variations in the maximum ECL emission when the coreactant concentrations of trimethylammonioethanethiol (red) and tripropylamine (blue) were changed. Trimethylammonioethanethiol was accumulated for 30 min, then washed with buffer before the measurements. Tripropylamine was simultaneously oxidized with 2 mM $Ru(bpy)_3^{2+}$.

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6 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

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