Electrochemiluminescence of Luminol Generated at Self-Assembled Monolayer of Ferrocenylalkanethiol on Gold Electrode

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Self-assembled monolayer of ferrocenylundecanethiol on gold electrode catalyzed electro-oxidative chemiluminescence from a luminol/hydrogen peroxide system.

A luminol-peroxide chemiluminescent system has attracted particular interests in the field of bioanalytical chemistry. For generating the light emission, the oxidation of luminol proceeds for the first step. Metal ions and organometallic compounds are known to catalyze the oxidation of luminol with peroxide in strong alkaline solution (pH 10–13).[1–3] Enzymes, such as peroxidase and catalase, can catalyze the oxidation even in weak basic media, although their stability is rather poor.[4,5]

In this work, we propose an electrochemically-controllable (i.e., potential-dependent) chemiluminescence system by using a gold electrode modified with ferrocene-terminated alkanethiol as the catalyst for oxidizing luminol. Ferrocenylalkanethiols have been known to form chemically/physically stable, well-packed self-assembled monolayer on gold surfaces.[6–8] The surface attached ferrocene moieties were electrochemically oxidized to form ferricinium ones, which catalyzed the oxidation of luminol effectively. The modified electrode was stable enough for the repetitive use.

3-Aminophthalhydrazide (luminol, Aldrich), hydrogen peroxide (30%, Wako Pure Chemicals) and 11-ferrocenyl-1-undecanethiol (FcC11SH, Dojin) were used without further purification. These reagents and the reagents for electrolyte/buffer solution were the extra pure grade. Millipore water (Milli-Q SP, Millipore Co.) was used to prepare electrolyte/buffer solution.

A vacuum deposited gold substrate (thickness: 100 nm, on cleaned slide glass pre-deposited with 1 nm of Cr) was used as working electrode. Gold electrode was modified by dip-treatment in ethanol solution of FcC11SH (1 mM) for 1 h. After modification, the electrode was thoroughly washed with pure ethanol and water.

To perform electrochemical and fluorescence measurements simultaneously, a three-electrode electrochemical cell system was constructed in a standard quartz photospectrofluorometry cuvette. An Ag/AgCl (3 M NaCl solution) and Pt wire were used as reference and auxiliary electrodes, respectively. (Electro) chemiluminescence ((E)CL) measurements were carried out using spectrofluorometer (HITACHI F-4500). The ECL (λem: 425 nm) was detected with a slit width of 20 nm. Electrochemical and ECL measurements were recorded at room temperature in 0.1 M buffer solution containing 0.1 M $NaClO_4$, luminol (100 μM) and hydrogen peroxide (10 mM) with different pH. Luminol and hydrogen peroxide were mixed just before the experiments.

The FcC11SH modified gold electrode showed reversible redox waves around +400 mV in the buffer solution containing 0.1 M $NaClO_4$ (pH 8.2), corresponding to the oxidation and reduction of the surface attached ferrocenyl groups.[6] The charge in the anodic peak was 35 μC cm^{-2}, which is equivalent to 2.2×10^{14} adsorbed molecules cm^{-2}, that is slightly smaller than the maximum coverage (2.7×10^{14} molecules cm^{-2}) calculated by assuming hexagonal close packing of the FcC11SH.[7]

Figure 1 shows the potential dependence of chemiluminescence and linear sweep voltammogram of the FcC11SH modified gold measured in the solution containing luminol and hydrogen peroxide. In this current–potential curve, anodic peaks of oxidation of ferrocenyl group and catalytic oxidation of luminol in solution were observed from +400 mV to +600 mV. The anodic peak current around +400 mV became larger than that measured in solution without luminol. When the potential became more positive than +400 mV, at which the oxidation of attached ferrocene moiety occurred, light emission was observed (Figure 1(b)). The light intensity increased gradually as the electrode potential became positive. The light

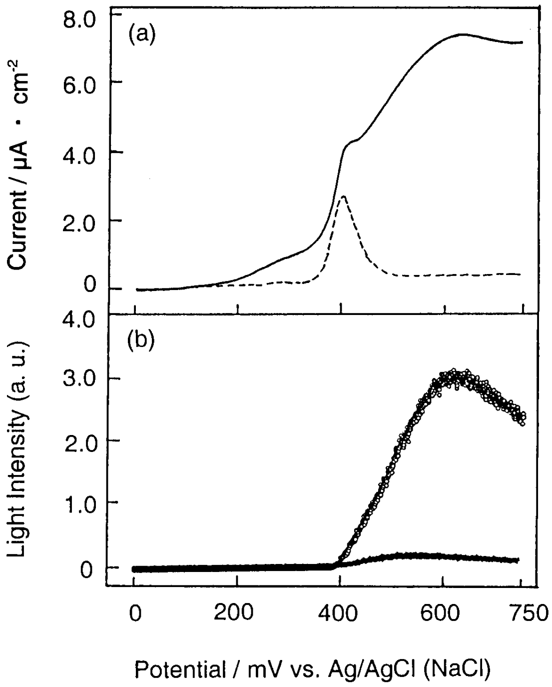

Figure 1. (a) Linear sweep voltammograms of FcC11SH modified gold electrode measured in electrolyte solution (0.1 M $NaClO_4$ / 0.1 M buffer solution, pH 8.2) with 100 μM luminol and 10 mM H_2O_2 (solid line) and without luminol and H_2O_2 (dotted line). Sweep rate : 2 mV/s. (b) ECL intensities of FcC11SH modified gold electrode (○) measured simultaneously with the linear sweep voltammograms. ECL intensity of unmodified gold electrode (◆).

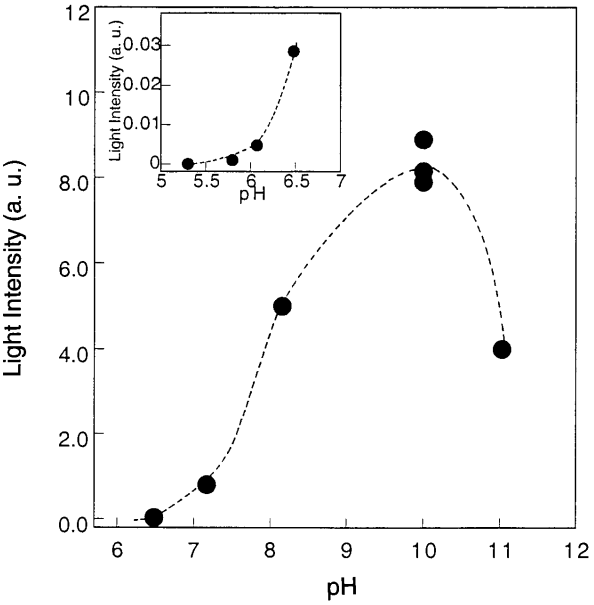

Figure 2. The pH dependence of chemiluminescence of FcC11SH modified gold electrode at +600 mV in buffered solution containing 0.1 M $NaClO_4$, 100 μM luminol and 10 mM H_2O_2. Inset: the pH dependence of chemiluminescence of same modified electrode measured in lower pH solution.

emission on the unmodified gold electrode was quite small in comparison with the FcC11SH modified gold as shown in this figure. These results indicated that ferrocenyl groups of the self-assembled monolayer on gold catalyzed the oxidation of luminol in solution effectively.

We also investigated the effect of pH on light emission. As shown in Figure 2, the light intensity became larger as the pH value in solution became higher. The light intensity reached the maximum at around pH 10. These observations were quite similar to the result for chemiluminescence property of luminol as reported before.[9] It is noteworthy that luminosity was observed even when the weak acid solution was used. In pH 5.8 solution, light intensity was also detected in this case, although the intensity was ca. 1/4000 of the response at pH 10.

We confirmed that ferricinium moiety participated to generate chemiluminescence reaction[10] as similar to the results using free ferrocene species.[11–13] The mechanisms of the electrochemiluminescence of luminol with H_2O_2 in aqueous alkaline solution have been presented.[14,15] It has been also reported that iron species could enhance greatly the luminol chemiluminescence reaction by oxidizing of both luminol and H_2O_2.[16,17] In our case, attached ferrocene group participated for catalytic oxidation of luminol (luminol anion). When the electrode potential was positive than the redox potential of ferrocene (~+400 mV), the oxidized ferrocenyl group catalyzed the oxidation of luminol. Then, reduced ferrocenyl group was re-oxidized to ferricinium cation on the modified electrode immediately.

Luminol-based assays have been carried out in alkaline solution since the quantum efficiency for generating chemiluminescent light of luminol is known to be increased with pH.[9] In this system, since the light intensity could also be detected in the weak acid solution (~pH 6), the H_2O_2-generation processes can be monitored by using the present modified electrode system in neutral moiety, which is of particular interest in achieving oxidase-coupled assays of the enzyme substrate. We have already succeeded in detecting glucose in the presence of glucose oxidase by using the ECL reaction on FcC11SH modified electrode system.[10]

References and Notes

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