Electrochemical Control of the Bioluminescence by Bacterial Luciferase Using Platinum-mesh Electrode

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Bioluminescence reaction by bacterial luciferase has been successfully controlled by a controlled-potential electrolysis using platinum-mesh electrode to regenerate the reduced form of flavin mononucleotide ($FMNH_2$), which is one of the substrates of the reaction. It was found that the regeneration of $FMNH_2$ is facilitated by the adsorbed hydrogen atoms formed on a platinum electrode surface in the presence of phosphate ions.

Bioluminescence of firefly luciferase (FFL) has been applied for a model system to assess the action of hydrophobic drugs like inhalation anesthetics on enzymatic reactions.[1–4] However, FFL is unstable and loses easily its enzymatic activity in aqueous solution. Bioluminescence of bacterial luciferase (BLuc) is one of the candidates replaceable to FFL system because of its high stability in aqueous solution.[5] BLuc is a flavin-dependent enzyme which catalyzes the bioluminescence reaction by using the reduced form of flavin mononucleotide ($FMNH_2$) as one of the substrates of the reaction, as in eq 1,

$$FMNH_2 + O_2 + C_{11}CHO \rightarrow FMN + H_2O + C_{11}COOH + \text{light} \quad (1)$$

where, $C_{11}CHO$ and $C_{11}COOH$ are the dodecylaldehyde and dodecanoic acid, respectively, in the present work.[6] However, $FMNH_2$ is readily oxidized by dissolved oxygen in ordinary atmospheric condition. Therefore, it is required to regenerate the $FMNH_2$ from flavinmononucleotide (FMN) for use of the BLuc system as the model system. In many reports on the BLuc system, chemically reducing agents such as sodium borohydride and sodium dithionate were used to reduce the FMN to $FMNH_2$.[7,8] Karatani et al. reported the electrochemical method for the reduction of FMN by using polymer-coated carbon electrode.[9] In the present study, we report the use of naked platinum-mesh electrode for the regeneration of $FMNH_2$ from FMN.

Bacterial luciferase (from *Vibrio fisheri*), flavinmononucleotide (FMN) sodium salt and dodecyl aldehyde ($C_{11}CHO$), were obtained from Sigma Chemical. The pH of the sample solutions were adjusted to 7.0 with phosphate buffer. $C_{11}CHO$ was dissolved in ethanol and an aliquot was added to the sample solution. The ethanol content of the sample solution was kept constant at 1.0% (v/v) in which it does not affect the luminescence intensity. Cyclic voltammogram (CV) was measured using conventional one-compartment glass cell with Pt-disk working electrode. The electrochemically generated bioluminescence (EBL) was measured using a home-made Diflon® cell having a quartz window and a Pt-mesh working electrode (80 mesh), which was equipped into a fluorescence spectrophotometer. In both the CV and EBL measurements, the reference and counter electrodes were Ag/AgCl (3.0 mol dm^{-3} NaCl) and Pt-wire, respectively. The sample solution of the EBL measurement consisted of FMN, $C_{11}CHO$, and BLuc in 50 mmol dm^{-3} phosphate buffer of pH 7.0.

Figure 1 shows the CVs of 10.0 mmol dm^{-3} phosphate buffer solution with and without 1.0 mmol dm^{-3} of FMN, respectively, measured with a Pt-disk electrode (1.6 mm diameter). The cathodic peak at around -0.5 V corresponds to the reduction of FMN to form $FMNH_2$. The peak at -0.7 V has been assigned to the reduction of H^+ ions to form adsorbed hydrogen atoms on a Pt electrode surface followed by the formation of hydrogen molecules.[10] The latter peak was observed only with Pt electrode and not observed with other electrodes such as gold and glassy carbon electrodes. In the presence of BLuc in the sample solution, the reduction peak of FMN was still observed with the Pt electrode, however, the peak was not observed with Au electrodes. This is because, in the case of Au electrodes, the BLuc molecules adsorb on the Au electrode surface and then hinder the electrode reaction of FMN.

Figure 2 shows the CV and corresponding EBL response of the solution containing 0.10 mmol dm^{-3} FMN, 0.10 mmol dm^{-3} $C_{11}CHO$, and 10 μmol dm^{-3} BLuc in 50 mmol dm^{-3} phosphate buffer observed by using the EBL cell. Although the reduction peak of the FMN in Figure 2 was small owing to the low FMN concentration in EBL solution, well-defined EBL signal was observed at the potential range of the reduction of FMN.

Figure 3 shows the time course profiles of the EBL signal during the controlled-potential electrolysis at several different potentials. Eventually, no EBL signal was observed at the electrolysis potential of -0.40 V (vs. Ag/AgCl) and the signal increased with increasing the electrolysis potential to more

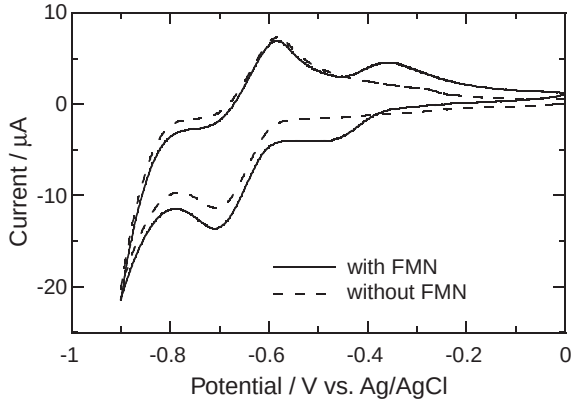

Figure 1. CVs of 10 mmol dm^{-3} phosphate buffer solution with and without 1.0 mmol dm^{-3} FMN. The solution was deaerated by N_2-bubbling. Potential scan rate was 50 mV s^{-1}.

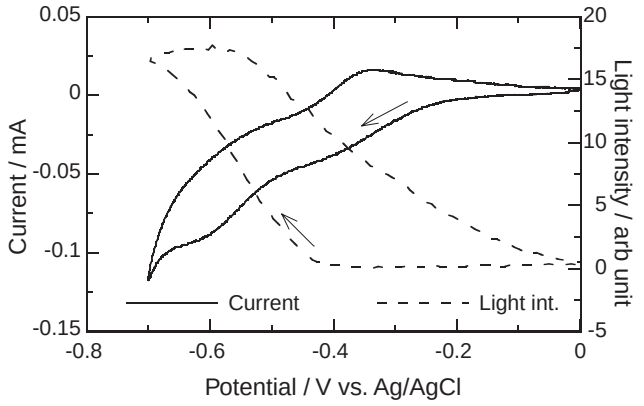

Figure 2. CV and corresponding EBL response of the solution containing 0.10 mmol dm^{-3} FMN, 0.10 mmol dm^{-3} $C_{11}CHO$, and 10 μmol dm^{-3} BLuc under aerobic condition. Potential scan rate was 5 mV s^{-1}.

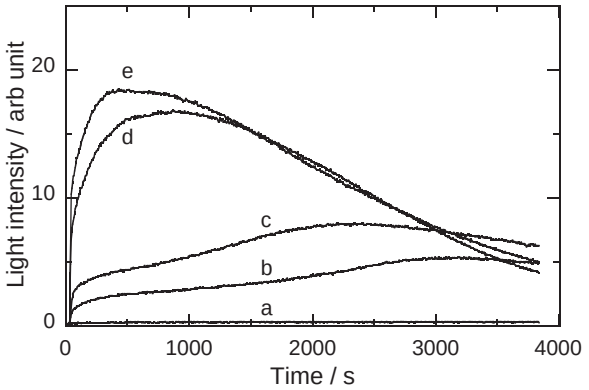

Figure 3. Time course profiles of the EBL signal during the controlled-potential electrolysis at the potential of (a) −0.40 V, (b) −0.47 V, (c) −0.50 V, (d) −0.60 V, and (e) −0.70 V.

negative values. It should be noted that two different types of the time course profile were observed depending on the electrolysis potential. In the EBL profiles of −0.47 and −0.50 V, the light intensity increased gradually with electrolysis time up to several tens of minutes. In the profiles of −0.60 and −0.70 V, sharp increase of the EBL signal was observed at the beginning of the electrolysis which followed by the gradual decrease. This decrease of the EBL signal after the sharp increase is probably due to the depletion of the $C_{11}CHO$ and/or dissolved oxygen which are consumed by the BLuc reaction. As previously reported by the present author,[10] the adsorbed hydrogen atoms (H_{ad}) are generated on a Pt electrode surface at the potential range lower than −0.6 V (Ag/AgCl) when the solution contains dihydrogen phosphate ions. In general, monoatomic state of hydrogen in aqueous solution is highly reactive with molecules and ions coexisting in solution. In the present case, the H_{ad} atoms are generated only at the electrolysis potential lower than −0.6 V as shown in Figures 1 and 2. Therefore, the two different types of the time-course EBL profiles are ascribed to the presence or absence of the participation of the H_{ad} atoms on the FMN reduction. The highly reactive H_{ad} atoms facilitate the reduction and successive protonation of FFM to form $FMNH_2$.

The EBL reaction requires the dissolved oxygen as one of the substrates of the reaction, as in eq 1. The dissolved oxygen is also reduced by the Pt electrode at the potential range of FMN reduction. It causes the depletion of the dissolved oxygen in the close vicinity of the electrode surface. Then, the EBL reaction does not proceed in this region of the $FMNH_2$ generation. To proceed the EBL reaction using dissolved oxygen, the $FNMH_2$ generated at the electrode surface should diffuse out of the electrical double layer of the electrode–solution interface. The H_{ad} atoms are not formed at the electrolysis potential of −0.47 and −0.50 V and hence the generation of $FMNH_2$ is rather limited as compared to the electrolysis at more negative potential. The gradual increase of the EBL signal with electrolysis time observed at the potentials of −0.47 and −0.50 V is then attributed to the limited generation of $FMNH_2$ and diffusion to the EBL reaction region. In the electrolysis at the potential of −0.60 and −0.70 V, in contrast, the H_{ad} atoms formed on a electrode surface facilitate the generation of $FMNH_2$ enough to initiate the EBL reaction even at the beginning of the electrolysis.

In conclusion, the EBL reaction of BLuc system was successfully controlled by using Pt-mesh electrode. The luminescence lasted for more than an hour by the regeneration of $FMNH_2$. The results showed that the H_{ad} atoms formed on a Pt electrode surface may critically affect the regeneration rate of $FMNH_2$. More detailed analysis on the action of H_{ad} atoms and the application of EBL reaction to drug assessment are now in progress in our laboratory.

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