Determination of Sub-nmol Hydrogen Peroxide by Electrochemiluminescence of Luminol in Aqueous Solution

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An electrochemiluminescence method was developed for determination of sub-nmol hydrogen peroxide in aqueous solution. Luminol was electrochemically oxidized, then reacted with hydrogen peroxide; it emitted light of the maximum wavelength of 436 nm with a half-life of less than 0.2 s from the electrode surface. In flow injection analysis a carrier solution (phosphate buffer, pH 7-9) containing 17 μ M luminol was delivered at the flow rate of 0.5 ml/min. The luminescence was detected by a photon counter in a spiral cell under the applied potential of 0.7 V vs. SCE. A straight line calibration plot was obtained with good reproducibility [RSD 1.6% ($n=5$)] in the concentration range of 0.066 to 0.6 nmol.

Keywords Electrochemiluminescence, flow injection analysis, hydrogen peroxide, luminol

The electrolytically generated chemiluminescence (electrochemiluminescence, ECL) is promising for sub-nmol analyses coupled with HPLC or FIA, as in the case of fluorescence or electrochemical detection. Blatchford *et al.*^{1,2} tried to detect some aromatic condensed-ring compounds by ECL in an aprotic mobile phase. But the applications were limited owing to the unstability of anion or cation radical in aqueous solution. One of the purposes of this investigation is to extend the capability to aqueous solutions.

Since hydrogen peroxide is produced by enzymatic reactions through uptake of oxygen, it may be useful for specific detection of the substrates. A lot of investigations have been reported for the determination of hydrogen peroxide at trace levels by electrochemical³ or fluorescence⁴ methods; sometimes the method is coupled with enzymatic reactions.⁵⁻⁷

Two-step oxidation was required for chemiluminescence of luminol as in the case of indole⁸ or tetracycline.⁹ Usually the oxidation of luminol by potassium ferricyanide was used for this purpose prior to the reaction with hydrogen peroxide. In the method presented in this report, the first oxidation step was replaced by an electrochemical oxidation, namely, luminol is electrochemically oxidized to diazaquinone, then reacts with hydrogen peroxide, and emits luminescence. Since the half life of the luminescence was very short, the light emission confined on the electrode surface allowed a high gain of photon counting. Moreover, this reaction proceeded in neutral aqueous solutions. The application of this method to the flow injection analysis (FIA) of hydrogen peroxide in neutral aqueous solutions at sub-nmol level is presented in this paper.

Experimental

Cyclic voltammetry (CV)

CV was measured by a Fuso Model 312 polarograph equipped with a Hokuto Model HB-104 function generator. The working electrode and counter electrode were two platinum wires: one of 0.5 mm ϕ and 6 mmL and the other of 0.5 mm ϕ and 3 cmL. SCE was used as the reference electrode. The solution temperature was controlled at $25 \pm 0.1^\circ\text{C}$.

Electrochemiluminescence

A Shimadzu spectrofluorophotometer, Model RF-510, was used for measurements of ECL spectra. An Aloka bioluminescence reader, Model BLR-102, was used for photon counting. For fundamental ECL measurements a platinum wire working electrode (0.5 mm ϕ , 5 cmL, spiral) was used in a Teflon cell (17 mm ϕ , 5 cm depth). The data acquisition time (integration time) was 30 s after the applied potential was imposed.

Flow injection analysis

Carrier solution was delivered by a pump, Toyosoda Kogyo Model CCPD, at a flow rate of 0.5 ml/min. A Teflon coil of 0.25 mm ϕ and 2 mL was provided in the stream for mixing of injected sample with luminol in the carrier solution.

An ECL flow-through cell, easily constructed by hand, was designed and used as shown in Fig. 1. The solution flowed through the spiral coil of a polyethylene tubing with inner diameter of 0.6 mm. A working electrode (a platinum wire of 0.5 mm ϕ and 10 cmL) was

Fig. 1 ECL cell for FIA.

inserted inside of the polyethylene tubing, and one end of the electrode was connected to the outside lead wire in a pool, where a counter electrode (platinum wire, 0.5 mm ϕ and 10 cmL) and a reference electrode (Ag/AgCl; -0.044 V vs. SCE) were also set up. The applied potential was controlled at 0.7 V vs. SCE. The spiral coil was set in front of the PM tube for photon counting.

Reagents

Luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) was purchased from Wako Pure Chemicals Co., and was purified as follows; The c.p. reagent was dissolved in warm 1 M sodium hydroxide solution, and 0.5 M hydrochloric acid was added slowly to neutralize the solution. The precipitate which formed was filtered and washed well with water. Then it was desiccated at room temperature under reduced pressure.¹⁰ All other chemicals were of analytical reagent grade, and were used without further purification.

Results and Discussion

Cyclic voltammetry of luminol

CV measurements were carried out to establish the optimum applied potential.

Luminol has two dissociation constants: 6.00 and 13.00 ¹¹; in a neutral solution it dissociates as a univalent anion. As shown in Fig. 2, the anodic process at Pt electrode in solution of pH 7.4 consisted of two steps: the first at 0.46 V and the second at 0.60 V, under the scan rate of 10 mV/s. Under the scan rate of 100 mV/s,

Fig. 2 CV of 2.0 mM luminol at Pt electrode in phosphate buffer (pH 7.4). Scan rate: (A) 10 mV/s, (B) 100 mV/s, (C) 200 mV/s.

the two peaks were still clearly separated, but at 200 mV/s they overlapped and an apparent one step oxidation was observed at 0.54 V under the scan rate greater than 200 mV/s. The oxidation product is diazaquinone^{12,13} as shown in the following. The pH dependence of the peak potential is expressed with the coefficient of -50 mV/pH. At a glassy carbon electrode, two-step oxidation was also shown at 0.48 and 0.60 V vs. SCE, and the third oxidation step, oxidation of amino group, appeared at about 1.10 V as a small hump in the CV pattern. This third step could not be observed well at the Pt electrode.

Electrochemiluminescence

The oxidation of luminol by Fe(III) or Cu(II) to form diazaquinone in the chemiluminescence procedure was replaced by the electrochemical oxidation, as mentioned in the previous section. Diazaquinone formed at the electrode surface is assumed to change into endoperoxide or hydroperoxide by the reaction with hydrogen peroxide, and an excited state of aminophthalic acid formed by fission of the diazaquinone ring emits light.¹¹

Figure 3 shows the dependence of ECL on the applied potential. It is seen that at potentials less than 0.5 V the luminescence could not be observed, while at more positive potentials the luminescence intensity was increased steeply. The noise level remarkably increased at potentials more positive than 0.7 V, and the S/N ratio showed a maximum at about 0.7 – 0.9 V, as shown in Fig. 3, curve B.

Fig. 3 Effect of applied potential on ECL. (A) luminescence intensity, (B) ratio of ECL versus background luminescence, 17 μ M luminol in phosphate buffer of pH 7.4, 1.32 nmol hydrogen peroxide.

The maximum wavelength of the emitted light at pH 7.4 was 436 nm, and in alkaline solution the maximum showed a blue shift. The half-life estimated by the decay curve of emission was less than 0.2 s.

When luminol concentration was changed from 10 nM to 1 mM under the constant experimental conditions, the luminescence intensity showed a maximum at 10–20 μ M luminol concentration. At high concentration of luminol the light emission was interfered by the adsorption of the oxidation product on the electrode surface. The pH dependence of the luminescence intensity showed a maximum at pH 7.4–10 as shown in Fig. 4. In solutions of pH less than 6, no luminescence was observed. At pH values higher than 8, background luminescence was increased; light emission could be observed even in the absence of applied potential at pH higher than 9.

Flow injection analysis

The following experiments were carried out to find out the optimal conditions of FIA: The carrier solution was phosphate buffer (pH 7.4) containing 17 μ M luminol, and the injected quantity of hydrogen peroxide was 1.32 nmol.

For the equipment used in this experiment the optimal flow rate was 0.3–0.5 ml/min. The flow rate of 0.5 ml/min was used throughout the experiments.

In consideration of the CV data, the potential as high as 0.7 V was applied to the working electrode. At this potential, the S/N ratio was fairly good and the noise level was sufficiently low.

When acetonitrile was added to the carrier solution, the luminescence intensity was increased at the concentration of 2–5%; its intensity quickly decreased at higher concentrations. The background luminescence was also increased by the addition of acetonitrile. A small quantity of methanol decreased the luminescence

Fig. 4 Effect of pH on ECL. (A) ECL at applied potential of 0.7 V; 17 μ M luminol and 1.32 nmol hydrogen peroxide, (B) background luminescence in the absence of applied potential; 17 μ M luminol and 1.32 μ mol hydrogen peroxide.

intensity drastically and even 0.1% methanol suppressed the light emission by 80%.

A good straight line calibration plot was obtained with the carrier solution (pH 9.2) containing 2% acetonitrile at the applied potential of 0.7 V vs. SCE. In this case the detection limit of hydrogen peroxide was as low as 30 pmol. When the pH was 7.4 and the carrier solution did not contain acetonitrile, a straight line calibration plot was obtained with the detection limit of 66 pmol and RSD as low as 1.6%.

It is noteworthy that the half-life of luminescence was very short in the case of luminol. For such a short decay time it is difficult to design an appropriate mixing device for chemiluminescence measurement. The photon counting of luminescence at the electrode surface, however, was especially feasible even for luminescence with very short life-time.

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