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Compact detection cell using optical fiber for sensitization and simplification of capillary electrophoresis–chemiluminescence detection

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Abstract

A new and simple chemiluminescence detection cell using optical fibers was designed for capillary electrophoresis. The cell was easily combined with capillary electrophoresis equipment and the system was operated without any tedious procedures. Luminol chemiluminescence was adapted for use with this detection cell. Hydrogen peroxide and catalysts were examined in detail with respect to their usage and concentration using the cell. The detection limit for luminol was $5.0 \cdot 10^{-10}$ *M* (14 amol; $S/N=3$), which was the most sensitive result reported so far. Also, a mixture of glycine, glycylglycine and glycylglycylglycine, which was labeled with isoluminol isothiocyanate, was subjected to the present system. They were sensitively detected and were baseline separated. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Detection, electrophoresis; Chemiluminescence detection; Instrumentation; Isoluminol isothiocyanate; Optical fiber; Glycine; Glycylglycine

1. Introduction

Over the past several years, capillary electrophoresis (CE) has been shown to be a powerful and efficient analytical separation technique. One of the major areas of study is the development of sensitive detection methods. On-column optical detection modes, such as UV absorption and fluorescence detection, are the most commonly used, on account of the extremely small sample zone and the small dimensions of the capillary in CE. Significantly more sensitive detection can be realized if the analytes can be used in conjunction with laser-induced fluorescence [1,2], amperometric [3] or radiometric [4] detection.

An alternative sensitive detection scheme is the use of chemiluminescence (CL) [5]. Although CL reactions lack selectivity, the CL detection system combined with separation methods can offer excellent analytical selectivity and sensitivity. CL has already been shown to be a highly sensitive detection method in both flow-injection analysis (FIA) and high-performance liquid chromatography (HPLC) [6–12].

Recently, the applicability of CL detection in CE has been successfully demonstrated. Several CL reagents, such as luminol [13–18], acridinium [19] and peroxyoxalate [20–24], have been utilized. We

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reported for the first time on the chemiluminescent detection of proteins separated by CE [20,22]. The proteins, labeled with fluorescent compounds, were quantitatively analyzed by the CE–CL detection method with detection limits of 10^{-8}–10^{-7} *M*. We also developed a CE apparatus with on-line CL detection of a luminol–H_2O_2 system [16], in which small detection limits of 10^{-9}–10^{-10} *M* were obtained for transition metal ion and hemoglobin. Last year, we reported on a CE system that was equipped with electrogenerated CL of Ru(II) complex [25].

Although research into combining CE with a CL detection system has increased in recent years, it is still problematic. Most of the CE–CL detectors reported so far, except for a few systems mentioned below, have involved variations of a postcapillary reactor to mix reagents. The reactors require the insertion of one capillary (separation capillary) into another larger capillary (reaction/detection capillary). The procedures are manually intensive and cumbersome, and it is difficult to reproducibly control reagent concentrations at the reactor. The separation capillary also has to be etched with hydrofluoric acid when reaction columns with relatively small inner diameters are used. Furthermore, the reagents must be passed through the larger capillary at an extremely small flow-rate by high performance and very expensive pumps.

Obviously, compared with other detection modes that are widely incorporated in CE, CL detection is an evolving technique. Therefore, advances should focus on the development of new detectors that are simpler instrumentally than existing systems. Zhao et al. [14] proposed the use of a sheath flow cuvette as a postcolumn reactor in CE. Daddo et al. [18] developed a CL detector interface that can be used with CE and involves the use of an optical fiber. Gilman et al. [26] also utilized an optical fiber to transport a CL signal in a CE system with an electrogenerated CL detector.

In this study, a novel compact CL detection cell was designed for CE. The detection cell, which was made of PTFE, was equipped with an optical fiber, a fused-silica capillary and a grounding electrode. The cell could be easily combined with CE equipment without any complex construction or expensive implements, and CE–CL detection was used for measurement without the need for any tedious procedures or special techniques. The CL light generated at the capillary outlet was transported by an optical fiber to a photomultiplier tube (PMT). In general, CL detection used not only for CE but also for FIA and HPLC requires detailed examination of reagent concentration, mixing order and the method of mixing to give sensitive and reproducible results and, consequently, these data sometimes offer unexpected and interesting findings. Here, reagent concentration and the mixing procedure for hydrogen peroxide (H_2O_2) and catalysts were investigated in detail. Luminol, as an analyte, gave a small detection limit, which was lower than any reported previously in CE–CL detection [13,14,18,26]. A sample mixture labeled with isoluminol isothiocyanate (ILITC) was also successfully separated and detected. Results for the sensitivity, linearity, resolution and reproducibility are also presented. The present CE–CL system might be expected to become a really practical CL detection system for CE.

2. Experimental

2.1. Reagents

All reagents used were commercially available and of analytical grade. Ion-exchanged water was distilled before use. Luminol, microperoxidase, glycine and glycylglycylglycine were purchased from Sigma. Glycylglycine was purchased from Peptide Institute, and isoluminol isothiocyanate and potassium ferricyanide were from Tokyo Chemical Industry and Wako, respectively.

2.2. Labeling procedure

Labeling using ILITC was carried out according to the procedures described in earlier reports [14,27]. A definite amount of amino acid or oligopeptide (micromolar order) was added to a microvessel and dissolved with 100 μl of water–triethylamine (95:5, v/v). The solution was subjected to ultrasonication for 1 min and then left in the dark for 20 min, while being vortex-mixed. The residue that was obtained by evaporation from the solution was redissolved in 10 m*M* phosphate buffer (pH 10.8) to give ILITC-labeled sample.

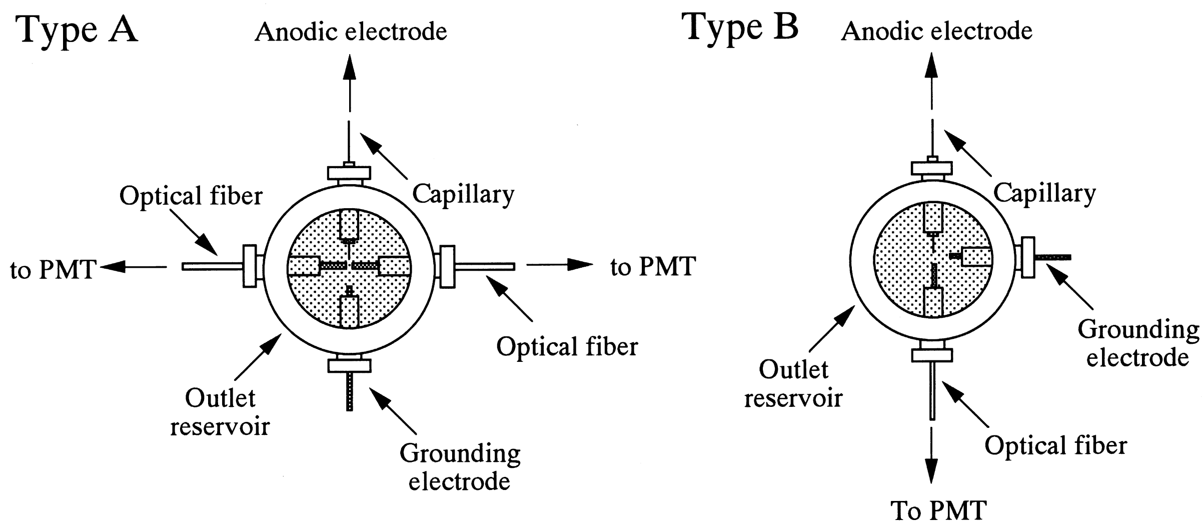

Fig. 1. Schematic diagram of the CL detection cells.

2.3. Chemiluminescence detection cell

A schematic diagram of two types of CL detection cell (A and B) is shown in Fig. 1. The detection cells were made of PTFE, which had a 4-cm outer diameter, and inner volumes of 2.5 and 8 ml. An optical fiber (with a core diameter of 2 mm; PGR-FB2000, Toray Industries), a fused-silica capillary (GL Sciences) and a platinum wire (as the grounding electrode) were fixed to the outlet reservoir. That is, the cell also worked as an outlet reservoir including an electrolyte. The end of the capillary was burned to strip 1–2 mm of the polyimide-coating off. Type A in Fig. 1 consisted of two optical fibers that were positioned at right angles to the inserted capillary. The distance between both optical fibers was 1.00 ± 0.05 mm (the distances from the center cell to the capillary and the optical fiber were 1.0 and 0.5 mm, respectively). The other type, type B, consisted of one optical fiber, which was set up straight to the capillary end with a space of 0.3 ± 0.05 mm between them. In both cells, the grounding electrode was kept at a distance of more than 8 mm from the center of the cell. As analytes emerged from the capillary, they reacted with reagents to produce visible light. The CL light was captured by the optical fibers.

2.4. Capillary electrophoresis apparatus and procedure

A previously unused capillary tube with an I.D. of 50 or 75 μm was treated with 1.0 *M* sodium hydroxide for 10 min and then washed with distilled water. A high voltage (12 kV) was applied to the electrodes using a d.c. power supply (Model HCZE-30PNO. 25, Matsusada Precision Devices). A luminol and H_2O_2 CL system was used together with microperoxidase or potassium ferricyanide as the catalyst. Luminol, ILITC, H_2O_2 and the catalyst were dissolved in 10 m*M* phosphate buffer, pH 10.8, which was used as a migration buffer. Stock solutions of the analytes (luminol, ILITC, ILITC-labeled compounds) were also prepared in the phosphate buffer and diluted as needed. Sample injections were performed by gravity for 4 or 10 s at a height of 20 cm. A sample migrated in the electrolyte towards the CL detection cell and mixed with the reagents. The resulting CL at the capillary outlet was transported by optical fiber to a PMT (Model R464, Hamamatsu). The output from the PMT fed a photon counter (Model C1230, Hamamastu) connected to an integrator (Chromatopac C-R6A, Shimadzu) to produce electropherograms.

The H_2O_2 and catalyst (microperoxidase and potassium ferricyanide) were used as follows: Usage A; H_2O_2 was added to the inlet reservoir and migrated to the CL detection cell with the electrolyte, and catalyst was added to the outlet reservoir (the CL detection cell) and, in reverse, usage B; catalyst was added to the inlet reservoir and H_2O_2 to the outlet reservoir.

3. Results and discussion

3.1. Design of chemiluminescence detection cell (types A and B)

The optimum conditions with respect to the capillary, the optical fiber and the ground electrode in both detection cells were described in Section 2, and were determined in terms of detection sensitivity for both types of detection cell. Certainly, the conditions significantly affected CL intensity. However, the setups were easily performed within the allowable range (±0.05 mm) and the data were obtained with good reproducibility. The performances of the two types of CL detection cell shown in Fig. 1 (types A and B) were compared under the following conditions: 100 m*M* H_2O_2 was added to the inlet reservoir and 25 m*M* potassium ferricyanide was added to the outlet. Luminol (as an analyte) migrated and the CL signal was observed. The systems using types A and B provided the electropherograms of Fig. 2a and b, respectively. Luminol concentrations

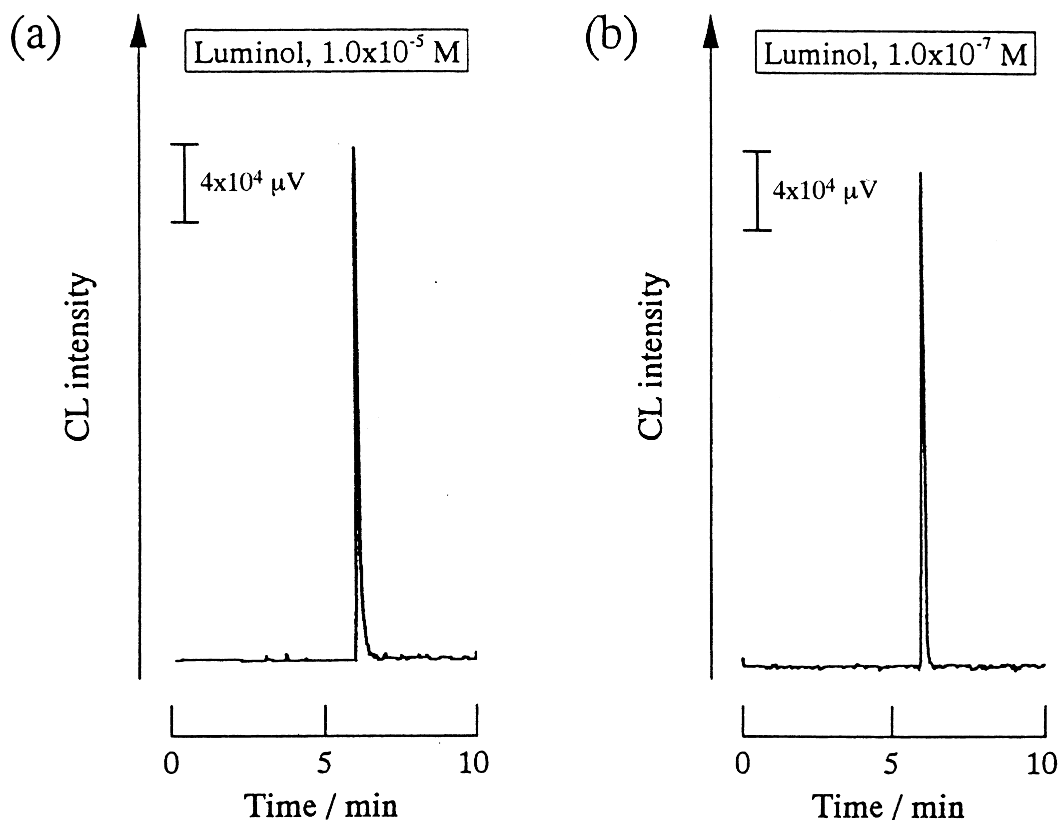

Fig. 2. Electropherograms of luminol obtained using CE–CL detection system of (a) type A and (b) type B. The column dimensions were 50 cm×75 μm I.D. Both the inlet and the outlet reservoirs contained 10 m*M* sodium phosphate (pH 10.8). In addition, 100 m*M* H_2O_2 was added to the inlet reservoir and 25 m*M* potassium ferricyanide was added to the outlet reservoir. The separation voltage was 12 kV. Sample injection was performed by gravity for 10 s (at a height of 20 cm). The luminol concentrations used for (a) and (b) were $1.0 \cdot 10^{-5}$ and $1.0 \cdot 10^{-7}$ *M*, respectively.

were (a) $1.0 \cdot 10^{-5}$ and (b) $1.0 \cdot 10^{-7}$ *M*. From the data, the type B system was found to be about 100 times more sensitive than type A.

It is interesting for us that type B, consisting of only one optical fiber, gave better results than type A, which consisted of two optical fibers. The following explanation, however, might be reasonable to understand the experimental results. First, one has to take the set-up conditions at the detection region limited by optical fiber into consideration; the optical fiber diameter (2 mm), the capillary inner diameter (75 μm) and the distance between the fiber and the capillary (0.3 mm). Electrolyte eluted to the region by electroosmotic flow with a rather rapid rate (>2.0 mm s^{-1}). In the case of type A, luminol that emerged from the capillary outlet would pass through at the detection region without any obstacles in a comparatively short time. The mixing that took place there must not have been sufficient for the luminol and CL reagent to mix well and generate large CL. On the other hand, in the case of type B, luminol that emerged from the capillary would immediately collide with the cross-section of the optical fiber because the distance between the capillary and the optical fiber is extremely short. The collision might bring about an effective mixing between luminol and CL reagent and thus generate a large CL signal. Consequently, the positioning of the fiber and the capillary in type B would lead to higher detection sensitivity than that of type A.

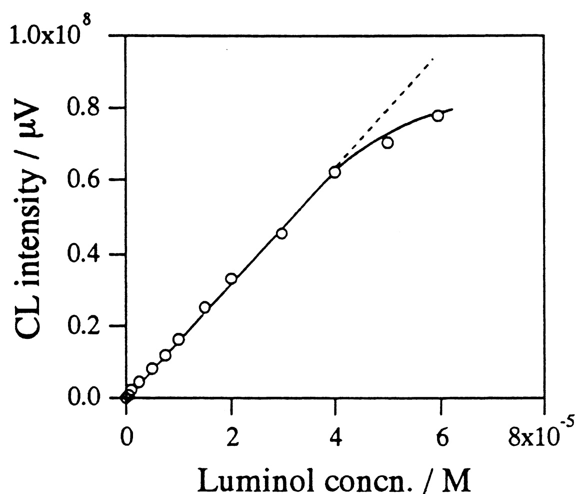

Fig. 3. Calibration curve for luminol using the CE–CL detector of type B. The experiments were carried out under the same conditions as those described in Fig. 2. The electrolyte in the outlet reservoir was exchanged at each measurement.

The calibration curve for luminol was obtained using the CE–CL detection system of type B (Fig. 3). The detection limit ($S/N=3$) was $7.0 \cdot 10^{-9}$ *M* (200 amol) and the relationship was found to be linear over three orders of magnitude (up to $4.0 \cdot 10^{-5}$ *M*). The theoretical plate numbers were 1200–1800. In spite of the initial data, they seemed to be equal to or better than the best results for luminol reported to date with CE–CL detection [13,14,18,26]. All further experiments were carried out using the CL detection cell of type B.

3.2. Chemistry of the chemiluminescence reaction in the cell

3.2.1. Examination of usage A

Much information concerning reagent concentration, mixing order, method of mixing, etc. has been reported for CL detection in conjunction with FIA and HPLC for the establishment of a highly sensitive and reproducible system. However, there seems to have been a paucity of information about CL detection in CE, until now. Luminol CL generally requires that luminol or its derivatives are mixed with H_2O_2 and catalyst. If the three components are introduced as separate streams, the peroxide is destroyed by the catalyst before reacting with luminol or its derivatives, and no CL is observed. First, in order to provide useful information on connecting the CE system with the CL detector, the way to mix H_2O_2 and catalyst was examined by the two usage procedures (A and B) described in Section 2. Microperoxidase [16,28,29] and potassium ferricyanide [30] were used as catalysts. Hydrogen peroxide and the catalyst were added to the inlet and outlet reservoirs, respectively.

The luminol signal, which had relatively high sensitivity, is shown in Fig. 2. The electropherogram was obtained using 100 m*M* H_2O_2 in the inlet and 25 m*M* potassium ferricyanide in the outlet reservoir. However, the CL signal decreased markedly in a continuous injection analysis and, finally, disap-

peared. The cause of the decrease in CL was investigated as follows.

First, decomposition of H_2O_2 due to electrolytic oxidation at the anodic electrode, about which there were some comments in an earlier report [17], should be considered. The results of the first series of measurements can be seen in Fig. 4. After finishing the first series of measurements, the electrolyte in the outlet was exchanged for a new one, with the electrolyte in the inlet reservoir being left as it was. Then, the second series of measurements was performed as well as the first series of measurements. Each of the series included the injection of luminol ten times and each measurement took 10 min. The curve of the second series was fitted to that of the first, as shown in Fig. 4. The cause of the decrease in CL in repeated injections probably is not due to the decomposition of H_2O_2.

Next, the CL intensity for repeated injections of luminol at various H_2O_2 concentrations (0.1, 0.5, 5 and 100 m*M*) in the inlet reservoir was examined

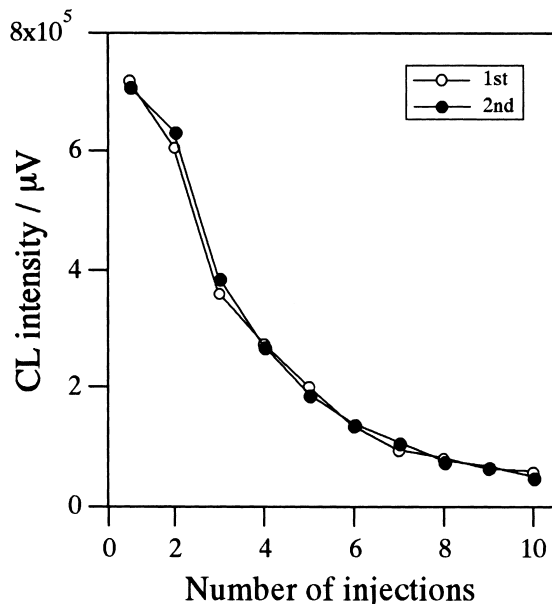

Fig. 4. CL intensity of luminol for the repeated injection experiment; Usage A: (○) the first series and (●) the second series experiments. The experiments were carried out under the same conditions as described in Fig. 2, except that the concentration of luminol was $1.5 \cdot 10^{-7}$ *M*. The electrolyte in the outlet reservoir was exchanged after the first series experiment followed by the second series experiment.

while maintaining a constant potassium ferricyanide concentration (25 m*M*). Although the rate of decrease in CL intensity became smaller at lower concentrations of H_2O_2, the CL decrease did not completely disappear, even at the lowest concentration of H_2O_2 (0.1 m*M*). At 0.1 m*M* H_2O_2, the CL intensity for the tenth injection was within about 40% of the first one. Instead of potassium ferricyanide, microperoxidase was used as another catalyst. Similar to the result with potassium ferricyanide, the CL intensity decreased with increasing numbers of sample injection. The tendency of the CL to decrease appeared marked at high concentration of H_2O_2 as well as when potassium ferricyanide was used as the catalyst.

The volume of electrolyte eluted from the capillary to the outlet for one measurement was only about 0.1% of the whole volume of the outlet reservoir. It seems strange that the small electrolyte volume eluted from the capillary undoubtedly brought about the decrease in the CL of luminol. However, when the reaction process of CL (catalyst first attacks H_2O_2 to generate active oxygen, followed by reaction of the active oxygen with luminol) is taken into consideration, the following explanation may be the reason for the decrease in CL. A little reaction product might more or less interact with catalyst in the cell to decrease the catalytic activity, so that the active oxygen necessary for exciting luminol would not be generated sufficiently. However, these analytical conditions (usage A) were not found to be useful.

3.2.2. Examination of usage B

The CL intensity decreased gradually when H_2O_2 was added to the inlet reservoir, as described above. It is also known that it is difficult to degas H_2O_2 solutions, and bubble formation in the separation capillary is problematic. In addition, the presence of a relatively high concentration of H_2O_2 would perturb separations. Therefore, we examined usage B; the H_2O_2 was added to the outlet reservoir and the catalyst was added to the inlet.

No CL signal was observed when potassium ferricyanide was added to the inlet reservoir and migrated with the electrolyte solution in the capillary. The exact reason for this is not known. The potassium ferricyanide may not migrate in the elec-

trolyte to the CL detection cell. Ferricyanide ion has three minus charges and its ion size is comparatively small. The electrophoretic mobility of the anion may be large enough to migrate to the anodic electrode (capillary inlet) against electroosmotic flow. Therefore, the anion could not be introduced into the CL detection cell from the capillary, which might result in the absence of CL.

It was reported [14] that relatively low concentrations (micromolar) of enzymes such as microperoxidase in the capillary did not influence the separation in CE. Here, microperoxidase (4 μ*M*) was used as a catalyst in the inlet reservoir and migrated with the electrolyte in the capillary. On the other hand, 100 m*M* H_2O_2 was added to the outlet. The electropherogram of luminol had excellent efficiency, of 20 000–30 000, and high sensitivity (Fig. 5). Furthermore, the CL intensity of luminol did not change during repeated injection measurements, as shown in Fig. 6. The relative standard deviation of the CL intensity was 2.2%. The following experiments were carried out under the conditions of usage B using microperoxidase as the catalyst.

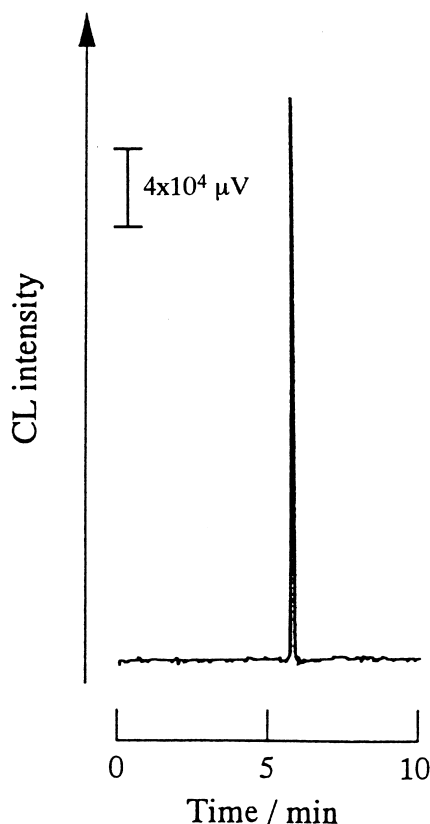

Fig. 5. Electropherogram of luminol obtained using the CE–CL detection system; Usage B. The experiments were carried out under the same conditions as described in Fig. 2, except for the following: 4 μ*M* microperoxidase was added to the inlet, 100 m*M* hydrogen peroxide was added to the outlet, and the concentration of luminol was $1.0\cdot10^{-8}$ *M*.

3.3. Sensitive detection of luminol

3.3.1. Optimization of regent concentration

Reagent concentration is also a very important factor for CL detection in CE, as well as in FIA and HPLC. The relationship between the microperoxidase concentration in the inlet reservoir and the CL intensity of luminol was examined using 100 m*M* H_2O_2 in the outlet reservoir (Fig. 7a). Maximum CL intensity appeared when the concentration of microperoxidase was around 2–4 μ*M*. The CL intensity decreased at high concentrations of microperoxidase. Since the repeated injection of luminol did not lead

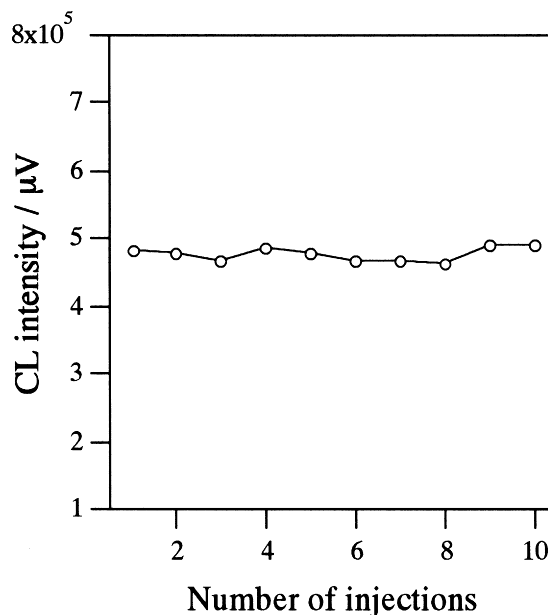

Fig. 6. CL intensity of luminol for the repeated injection experiment; Usage B. The experiments were carried out under the same conditions as described in Fig. 5, except that the concentration of luminol was $1.5\cdot10^{-8}$ *M*.

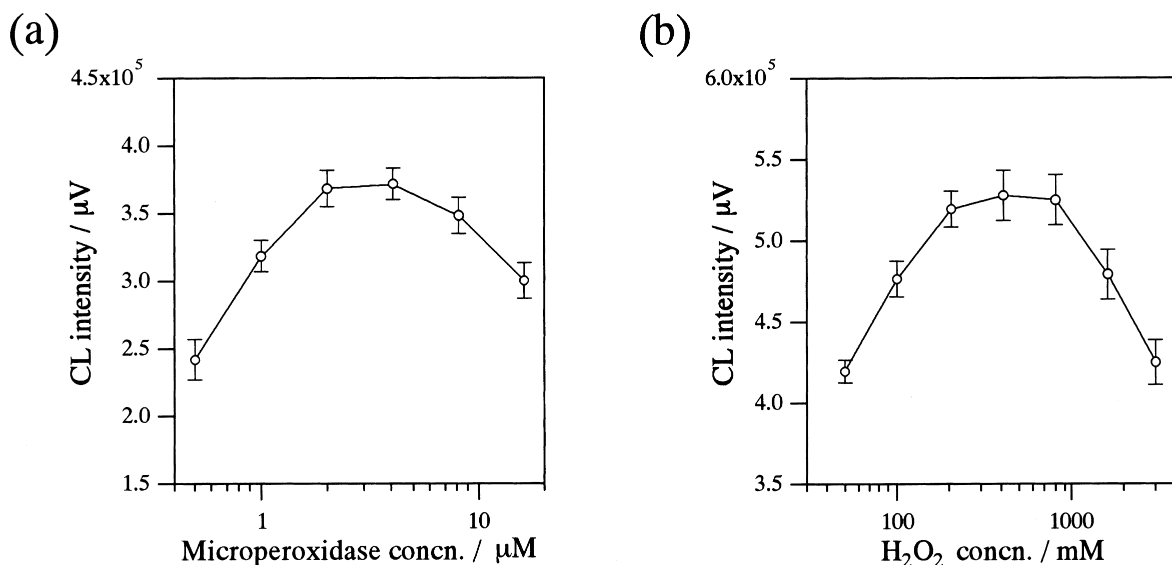

Fig. 7. Effect of (a) microperoxidase and (b) hydrogen peroxide concentrations on the CL intensity of luminol. Usage B. The experiments were carried out under the same conditions as described in Fig. 5 except that the concentrations of luminol used for (a) and (b) were $1.2 \cdot 10^{-8}$ and $1.8 \cdot 10^{-8}$ M, respectively. Measurements were carried out ten times at each concentration of microperoxidase and hydrogen peroxidase. Average values were plotted and one standard deviation (1σ) is indicated by error bars.

to any change in CL intensity, the decrease was not caused by the consumption of H_2O_2 in the presence of high concentrations of microperoxidase. Microperoxidase at high concentrations must interact or adhere to luminol in a capillary to a large extent. The microperoxidase around luminol would interfere with the contact between luminol and active oxygen, so that the CL intensity might decrease at high concentrations of microperoxidase.

3.3.2. Calibration curve for luminol

In addition, the relationship between the H_2O_2 concentration in the outlet reservoir and the CL intensity of luminol was examined with 4 μ*M* microperoxidase in the inlet (Fig. 7b). Maximum CL intensity was observed with 400–800 m*M* H_2O_2, where the relative standard deviation was 2.9%. The value increased slightly with increasing H_2O_2 concentration. At high concentrations of H_2O_2, some bubbles appeared on the inner wall of the outlet reservoir. The formation of bubbles may have brought about the increase in the relative standard deviation.

Calibration curves of luminol were examined under the optimized conditions described above; 4 μ*M* microperoxidase and 400 m*M* H_2O_2 (Fig. 8). The curve obtained using a capillary with a 50-μm I.D. represented the linear detection range, $1.7 \cdot 10^{-9}$–$5 \cdot 10^{-6}$ M (r=0.999); the detection limit (S/N=3),

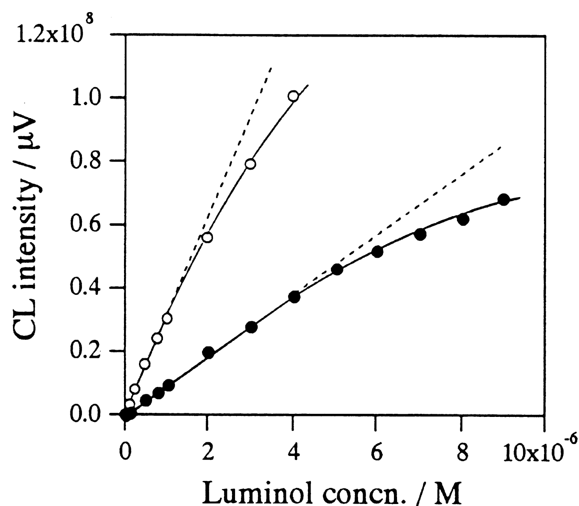

Fig. 8. Calibration curves for luminol using (○) 75 μm and (●) 50 μm I.D. capillaries. Usage B. The experiments were carried out under the same conditions as described in Fig. 5, except that 400 m*M* H_2O_2 was added to the outlet.

$1.7 \cdot 10^{-9}$ *M* (10 amol) and the theoretical plate numbers, 70 000–80 000. Using a 75 μm I.D. capillary, the linear detection range was $5.0 \cdot 10^{-10}$–$1.0 \cdot 10^{-6}$ *M* (r=0.999); the detection limit (S/N=3) was $5.0 \cdot 10^{-10}$ *M* (14 amol) and the theoretical plate numbers were 20 000–30 000. These detection limits were approximately five or six orders of magnitude lower than those obtained using absorbance. Daddo et al. [18] reported excellent design for CE–CL detection and derived the following data; detection limit of luminol, $2.0 \cdot 10^{-8}$ *M* (500 amol) and theoretical plate number, 10 000–20 000. The present system was also more sensitive for luminol than found using any other CE system equipped with a CL detection system reported before [13,14,18,26]. In the present system, the optical fiber was positioned straight at the face of the capillary outlet, with a small space between them. The positioning of the fiber must contribute to the effective capture of the small CL signal. In other words, the construction must lead to the relatively small reaction/detection zone and prevent the separation efficiency from being lowered due to band broadening and prevent the CL intensity being decreased at the capillary outlet.

3.4. Application for isoluminol isothiocyanate-labeled compounds

3.4.1. Optimization of reagent concentration

Many luminol derivatives have been widely researched as labeling reagents [8,14,27,31–33]. ILITC [14,27] is a commercially available reagent for labeling amino groups. Like other isothiocyanates, this reagent has potential application in protein sequencing. In this study, ILITC was used to label glycine, glycyglycine and glycylglycylglycine. Optimum concentrations of microperoxidase and H_2O_2 for ILITC and ILITC-labeled glycylglycine were investigated as follows.

The relationship between the microperoxidase concentration in the inlet reservoir and the CL intensity was examined at a constant H_2O_2 concentration (10 m*M*) in the outlet reservoir (Fig. 9a). The maximum CL intensity of ILITC was observed over a microperoxidase concentration of 5–8 μ*M*, where the relative standard deviation was 2.5%. On the other hand, the maximum CL of ILITC-labeled glycylglycine was found at concentrations of microperoxidase of around 15–25 μ*M* and the relative standard deviation was 2.2–2.8%.

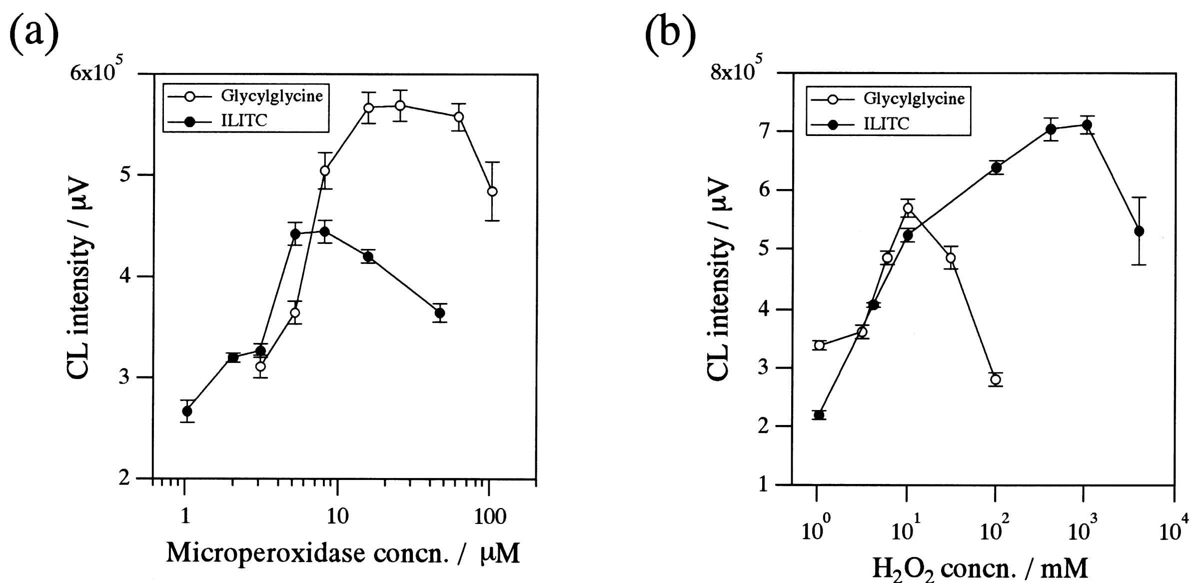

Fig. 9. Effect of (a) microperoxidase and (b) hydrogen peroxidase concentrations on CL intensity of ILITC and ILITC-labeled glycylglycine. Usage B. The experiments were carried out under the same conditions as described in Fig. 5, except that 10 m*M* H_2O_2 was added to the outlet and 20 μM microperoxidase was added to the inlet. Measurements were carried out six times at each concentration of microperoxidase and hydrogen peroxidase. Their average values were plotted and one standard deviation (1σ) is indicated by error bars.

The relationship between the H_2O_2 concentration in the outlet reservoir and CL intensity was examined at the recommended microperoxidase concentration (20 μM) in the inlet reservoir (Fig. 9b). The maximum Cl intensity of ILITC appeared at about 400–1000 m*M* H_2O_2, where the relative standard deviation was 2.3–2.5%. On the other hand, the maximum CL of ILITC-labeled glycylglycine was found at about 10 m*M* H_2O_2 and the relative standard deviation was 2.8%. An extremely large standard deviation was observed at high H_2O_2 concentrations (4 *M*). Bubble formation would interfere with measurement of a stable CL signal.

As described above, the optimum concentrations of microperoxidase and H_2O_2 for ILITC were similar to those for luminol. On the other hand, the optimum concentrations for ILITC-labeled glycylglycine were comparatively different from those for luminol. The labeling procedure would alter the CL quantum yield and rate kinetics [14].

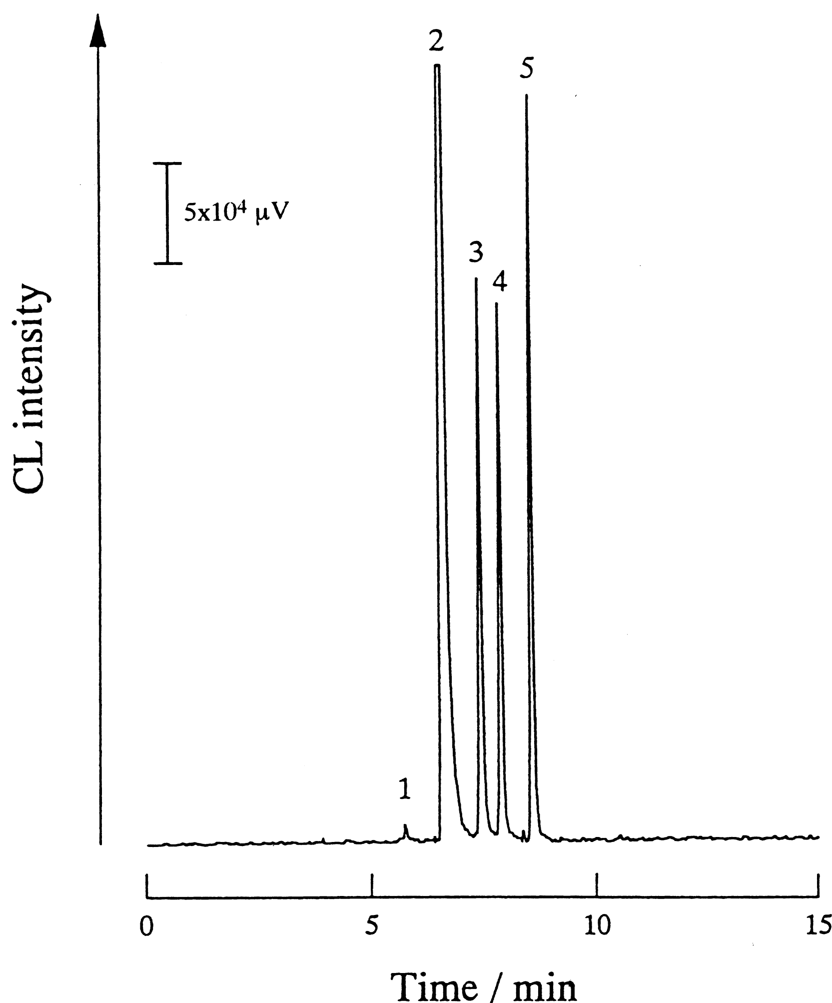

Fig. 10. Separation of ILITC-labeled compounds. Usage B. The experiments were carried out under the same conditions as in Fig. 6, except for the followingL 20 μ*M* microperoxidase was added to the inlet and 10 m*M* H_2O_2 to the outlet, and the column dimensions were 50 μm I.D. and 50 cm long. Peak identification: (1) hydrolyzed ILITC, (2) ILITC, (3) ILITC-labeled glycylglycylglycine, (4) ILITC-labeled glycylglycine and (5) ILITC-labeled glycine.

Table 1
Detection limits, linear detection ranges, correlation coefficient and theoretical plate numbers of ILITC-labeled compounds when the 50 μm I.D. capillary was used

Injection time (s)	ILITC-labeled compounds	Detection limit (S/N=3)		Linear detection range (M)	Correlation coefficient	Theoretical plate number
		Concentration (M)	Mass (amol)			
10	Glycylgycylglycine	$1.1\cdot10^{-7}$	620	$1.1\cdot10^{-7}$–$5.0\cdot10^{-6}$	0.999	40 000–50 000
	Glycylglycine	$1.1\cdot10^{-7}$	620	$1.1\cdot10^{-7}$–$6.0\cdot10^{-6}$	0.999	50 000–70 000
	Glycine	$8.1\cdot10^{-8}$	460	$8.1\cdot10^{-8}$–$6.0\cdot10^{-6}$	0.998	70 000–90 000
4	Glycylgycylglycine	$2.2\cdot10^{-7}$	500	$2.2\cdot10^{-7}$–$3.5\cdot10^{-6}$	0.995	80 000–90 000
	Glycylglycine	$2.1\cdot10^{-7}$	470	$2.1\cdot10^{-7}$–$3.5\cdot10^{-6}$	0.992	80 000–100 000
	Glycine	$1.3\cdot10^{-7}$	290	$1.3\cdot10^{-7}$–$6.0\cdot10^{-6}$	0.999	100 000–130 000

3.4.2. Separation and detection of labeled compounds

A mixture of glycine, glycylglycine and glycylglycylglycine, which were labeled with ILITC, was subjected to the present CE–CL detection system. The electropherogram obtained is shown in Fig. 10. The compounds were sensitively determined and completely baseline separated. The small peak at about 6 min may be due to hydrolyzed ILITC. The reactivity of ILITC was estimated to be about 50% for all samples, judging from the calibration curves for ILITC.

The detection limit, the linear detection range, the correlation coefficient and the theoretical plate number for the ILITC-labeled compounds are summarized in Tables 1 and 2, which correspond to the data using 50 and 75 μmm I.D. capillaries, respectively.

For example, in the case of the 75 μm capillary and a 10-s injection time, ILITC-labeled glycine was determined over the linear detection range of $2.3\cdot10^{-8}$–$8.0\cdot10^{-6}$ M (r=0.999), with a detection limit of 670 amol. Furthermore, good separation efficiency was expressed by the theoretical plate numbers, i.e. 20 000–30 000. Judging from these data, we believe that the present CE–CL detection system features excellent performance for selectivity and sensitivity. However, it must be noted that these detection limits are for the isoluminol thiocarbamyl derivatives, that is, the detection limits are for our present CL instrument. Derivatization for the trace amount of sample is difficult. We have not optimized the reaction chemistry to label samples of low concentration.

4. Conclusion

Recently, the applicability of CL detection in CE

Table 2
Detection limits, linear detection ranges, correlation coefficient and theoretical plate numbers of ILITC-labeled compounds when the 75 μm I.D. capillary was used

Injection time (s)	ILITC-labeled compounds	Detection limit (S/N=3)		Linear detection range (M)	Correlation coefficient	Theoretical plate number
		Concentration (M)	Mass (amol)			
10	Glycylgycylglycine	$12.8\cdot10^{-8}$	810	$2.8\cdot10^{-8}$–$6.0\cdot10^{-6}$	0.999	20 000–30 000
	Glycylglycine	$3.2\cdot10^{-8}$	930	$3.2\cdot10^{-8}$–$3.5\cdot10^{-6}$	0.999	20 000–30 000
	Glycine	$2.3\cdot10^{-8}$	670	$2.3\cdot10^{-8}$–$8.0\cdot10^{-6}$	0.999	20 000–30 000
4	Glycylgycylglycine	$25.2\cdot10^{-8}$	600	$25.2\cdot10^{-8}$–$5.0\cdot10^{-6}$	0.998	30 000–40 000
	Glycylglycine	$5.1\cdot10^{-8}$	590	$25.1\cdot10^{-8}$–$5.0\cdot10^{-6}$	0.999	40 000–600 000
	Glycine	$3.8\cdot10^{-7}$	440	$3.8\cdot10^{-8}$–$68.0\cdot10^{-6}$	0.998	50 000–70 000

has been successfully demonstrated. Compared with other detection modes widely incorporated in CE, CL detection is obviously an evolving technique. However, coupling a separation device with a CL detector is still problematic. Advances should focus on the development of new detectors that are instrumentally simpler than existing systems. In this study, a new, very compact CL detection cell was designed for CE. The cell could be easily combined with CE and used for measurement, without any complex construction, expensive implements, tedious procedures or special techniques. The luminol was eluted within 10 min with the detection limit of $5.0 \cdot 10^{-10}$ *M* (14 amol; $S/N=3$), which was approximately five or six orders of magnitude lower than those obtained using absorbance measurements. The present system was also more sensitive for luminol than any other CE method equipped with CL detection reported so far. A mixture labeled with ILITC was also subjected to the present system. They were sensitively determined and were completely baseline separated. The present system might be promising for numerous applications in fields such as environmental analysis and medicine on account of its high selectivity and sensitivity.

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