Capillary Electrophoresis with Absorption/Chemiluminescence Dual Detection System Using a Poyl(tetrafluoroethylene) Separation Capillary

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We developed a capillary electrophoresis system, incorporating absorption/chemiluminescence dual detection, with the use of a poly(tetrafluoroethylene) capillary as a separation tube. Chemiluminescence reaction of luminol-microperoxydase-hydrogen peroxide was adopted. Isoluminol isothiocyanate labeled-glycine, glycylglycine, and glycylglycylglycine were examined as model samples in the present system. These samples were first analyzed by absorption detection with on-capillary, followed by chemiluminescence detection with end-capillary. The absorption and chemiluminescence data were simultaneously obtained with on-line for quite the same samples, and they were easily and precisely compared with each other and discussed in terms of the migration times.

Key words: capillary electrophoresis, dual detection; luminol, absorption, poly(tetrafluoroethylene)

1. Introduction

A capillary electrophoresis (CE) has received much attention as a powerful separation method, where fused-silica capillaries are mainly used as a separation tube[1, 2]. A poly(tetrafluoroethylene) (PTFE) capillary is also widely used in the fields of not only analytical chemistry but also pharmaceutical chemistry and medicine[3, 4]. However, there have been few reports with regard to the CE taking advantage of PTFE capillary as a separation tube[5, 6]. The light scattering due to the crystallinity of PTFE causes a problem in sensitivity of CE equipped with on-capillary detection, such as absorption and fluorescence detection. The PTFE capillary seems to be rather matched to end-capillary detection, such as chemiluminescence (CL) detection.

In this study, we developed the CE system using a PTFE capillary as a separation tube that incorporated an absorption/CL dual-detection scheme. Both the absorption detection with on-capillary and chemiluminescence detection with end-capillary were successfully combined into a single system using one PTFE capillary to give a unique capillary electrophoretic system. The samples were first analyzed by absorption detection with on-capillary, followed by CL detection with end-capillary. We demonstrated that the present CE system provided interesting information in analyses of isoluminol isothiocyanate (ILITC)-labeled compounds.

2. Experimental

2.1 *Reagents*

All reagents used were of commercially available and analytical grade. Water was purified with Elix UV 3 (Millipore Co.). Microperoxidase, glycine (Gly), glycylglycine (GlyGly), glycylglycylglycine (GlyGlyGly), tryptophan (Try), phenylalanine (Phe), and tyrosine (Tyr) were purchased from Nacalai Tesque. ILITC and hydrogen peroxide solution (30wt%) were purchased from Wako Pure Chemical Industries, Ltd.

2.2 *Labeling procedure*

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Labeling with ILITC was performed as previously described[7, 8]. A known amount of amino acid or oligopeptide (on the order of micromoles) was added in conjunction with ILITC to a microvessel and dissolved in 100 μl of a mixture of water and triethylamine (95:5 volume ratio). The solution was subjected to ultrasonication for 1 min and then left in a dark place for 20 min with mixing by a vortex mixer. The residue obtained by evaporation from the solution was redissolved in 10 mM carbonate buffer (pH 10.8) to give a definite concentration of ILITC-labeled sample solution.

2.3 *CL detection*

A schematic diagram of the CL detector is shown in Fig. 1[9]. A batch-type CL detection cell was used in the CE system incorporating the absorption/CL dual detection. The detection cell was made of quartz and the inner volume was ca. 0.8 ml. The CL detection cell was enclosed in a small light-tight box together with a photomultiplier tube (H5783, Hamamatsu Photonics Co., Ltd.) to realize a compact CL detector. A PTFE capillary and a platinum wire as a grounding electrode were fixed to the detection cell; the cell also worked as an outlet reservoir including an electrolyte solution. The distance between the capillary end and the cell bottom was kept at about 1 mm. As analytes emerged from the capillary, they reacted with reagents to produce visible light. The CL light was detected with the photomultiplier tube located at the bottom of the cell.

2.4 *Analytical procedure*

The running buffer and the solution in the cell were prepared to detect ILITC and ILITC-labeled compounds as follows: A 10 mM carbonate buffer (pH 10.8) containing 4 μM microperoxidase was prepared as a running buffer and a 10 mM carbonate buffer (pH 10.8) containing 400 mM hydrogen peroxide was added to the CL detection cell (cell buffer). The running and cell buffers were exchanged for every measurement.

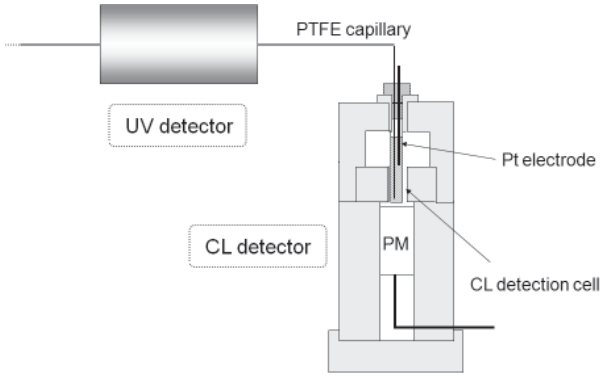

Fig. 1. Schematic diagram of CL detection in the CE.

A high voltage (10 kV) was applied to electrodes using a DC power supplier (Model HCZE-30P NO. 25, Matsusada Precision Devices Co., Ltd.). A PTFE (Yasaka Industries, Inc.) of 100 μm i.d., 200 μm o.d., and 90 cm length was used with effective lengthes of 70 cm for absorption detection (280 nm) and 90 cm for CL detection. Sample injection was performed by gravity for 10 s at a height of 20 cm. The sample migrated into the running buffer toward the CL detection cell and mixed with the reagents. The absorption detection was carried out on-capillary by a modified SPD-6A spectrophotometric detector (Shimadzu Co.), followed by end-capillary CL detection by the CL detector. The outputs from the detectors were fed to an integrator (Chromatopac C-R8A, Shimadzu Co.) to produce electropherograms.

3. Results and Discussion

3.1 *Properties of PTFE capillary in CE system*

The crystallinity of the PTFE causes the light scattering; the degree of the crystallinity is different, even though a litter, among the manufactured PTFE. In addion, impurities or additives in the PTFE must influence the characteristics of electroosmotic flow in CE. Taking into account these factors, as Macka et al. examined the optical properties and electroosmotic flow characteristics in CE[5], we carried out the following experiments relevant to the present PTFE capillary.

First, the PTFE capillary was fixed to the modified absorption detector. The capillary was then filled with water and absorption to the capillary tube was examined at 200-650 nm wavelength. The capillary showed a maximum extent of absorption at 200 nm and the absorption decreased with increasing wavelength. The absorption detection, particularly in the ultraviolet region, in CE was disturbed with the light scattering due to PTFE material.

Next, we examined the effect of pH (in an alkaline range from pH 7.3–10.8 for CL reaction) on the migration time of acetone as a neutral marker in CE with the absorption detection system (Fig. 2). The migration times became shorter with increasing pH values; the sufficient cathodic electroosmotic flow was observed in the alkaline solution that was required for CL reaction. The cathodic electroosmotic flow in the PTFE capillary may be explained by the presence of surface carboxylate groups formed by oxidation under the influence of UV light and/or ozone[10].

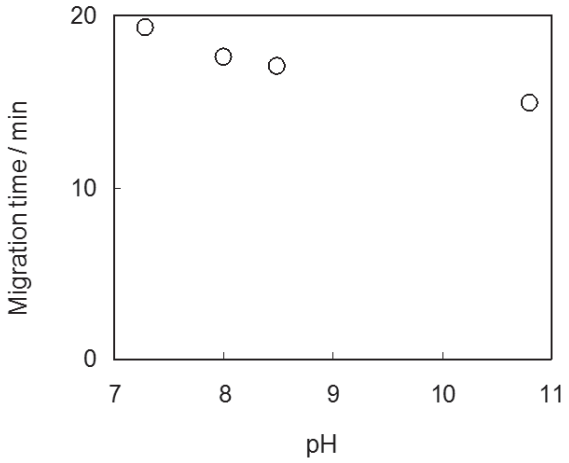

Fig. 2. Effect of pH on migration time of acetone as a neutral marker when using the CE-absorption detection.
Conditions: PTFE capillary of 100 μm i.d., effective length of 70 cm for absorption detection and total length of 90 cm; applied voltage, 10 kV; running buffer, 10 mM carbonate buffer (pH 10.8); and detection, 280 nm.

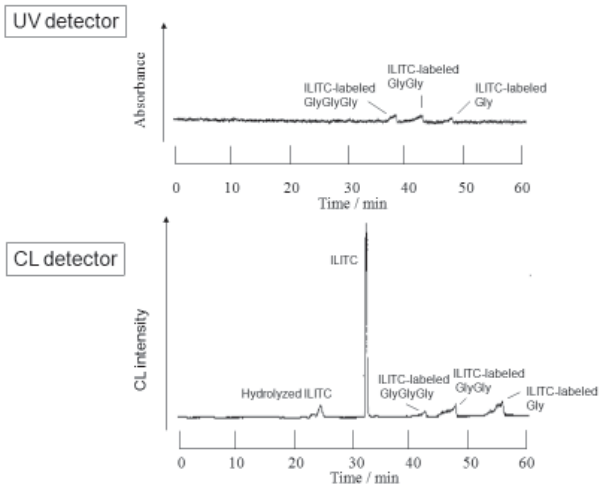

Fig. 3. Electropherograms of the mixtures of Try, Phe, and Tyr by the CE-absorption detection for pH 7.3 or 10.8 of the running buffer.
Conditions: PTFE capillary of 100 μm i.d., effective length of 70 cm for absorption detection and total length of 90 cm; applied voltage, 10 kV; reagent, 10 mM phosphate buffer (pH 7.3) or 10 mM carbonate buffer (pH 10.8); detection, 280 nm; and sample, 0.1 mM α-amino acids.

3.2 *Analysis of the mixture of α-amino acids with CE-absorption detection*

The mixtures of Try, Phe, and Tyr were subjected to the CE-absorption detection using the PTFE capillary. The obtained electropherograms are shown in Fig. 3. For pH 7.3 of running buffer, only single peak was observed. It was confirmed that the peak was due to the well-mixed behavior of Try, Phe, and Tyr; no separation was attained under the conditions. For pH 10.8 of the running buffer, on the other hand, Try and Phe were under detection at ca. 28 and 47 min, respectively, while Tyr was not detected within 2 h.

They are neutral α-amino acids that possess one amino group and one carboxyl group in a molecule. Amino group is protonated and carboxyl group is dissociated under the conditions of pH 7.3. Consequently, they were apparently neutral molecules in the capillary, which caused no separation in the CE. At pH 10.8 carboxyl group is dissociated to give negative charge on the molecule and amino group is not protonated. In addition, phenol group in Tyr must be dissociated to give negative charge. Based on their molecular weights, the ratio of charge to mass decreases in order of Try, Phe, and Tyr. As their electrophoretic mobilities turn toward the capillary inlet, migration times of Try, Phe, and Tyr must increase in the above

order. This sequence is consistent with the results shown in Fig. 3. The peak of Trp appeared faster than that of Phe on the electropherogram. Tyr was not detected; the electrohporetic mobility toward the capillary inlet must be larger than the electroosomotic flow. Also, the negative charge in relatively small molecules must bring about fronting in the obtained peaks.

3.3 *Analysis of the mixture of α-amino acid and oligopeptides with CE-absorption/CL detection*

ILITC is one of the reagents used to label amino groups. Like other isothiocyanates, this reagent has potential application in protein sequencing. Here, a mixed sample of Gly, GlyGly, and GlyGlyGly, all of which were labeled with ILITC, was analyzed in the present system. The resultant electropherograms are shown in Fig. 4. ILITC (free or excess ILITC), the labeled GlyGlyGly, GlyGly, and Gly were separated and detected in this order, although ILITC was not detected with absorption. At a running-buffer pH of 10.8, ILITC has a neutral charge and all of the labeled compounds would have negative charges due to the one carboxyl group in their molecules. The magnitude of electrophoretic mobility toward the inlet was Gly > GlyGLy > GlyGlyGly > ILITC, and the electroosmotic flow to the outlet was larger than the electrophoretic mobility. Thus, the migration sequence on the electropherogram, ILITC > GlyGlyGly > GlyGly > Gly was reasonably expected.

As shown in Fig. 4, ILITC was detected at a sufficient level with CL but not detected with absorption. It is known that CL detection can detect luminol or ILITC with much higher sensitivity than absorption detection[11]. However, in Fig. 4, ILITC-labeled compounds were observed with almost the same response intensities on the electropherograms for both detection manners, judging from their signal/noise ratios. The data immediately indicated a unique CL performance in which the CL quantum yield of ILITC became extremely low under the present conditions due to the labeling procedure. In order to improve the CL quantum of ILICL-labeled compounds, one may have to examine the analytical CL conditions for ILITC-labeled compounds, including pH, solvent, reagent concentrations, etc.

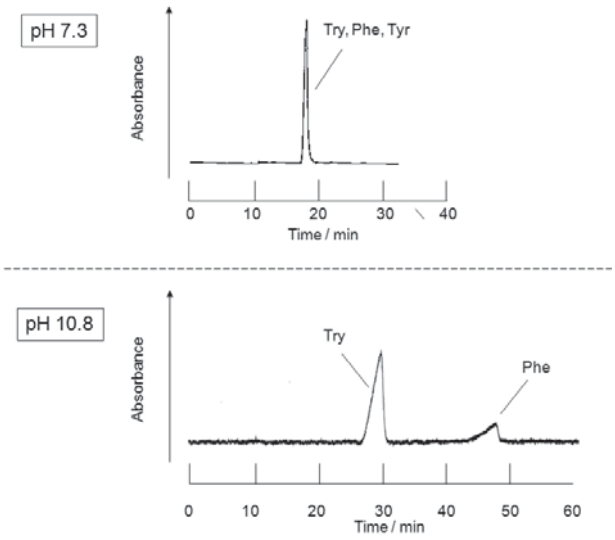

Fig. 4. Electropherograms of the mixture of ILICT and ILITC-labeled Gly, GlyGly, and GlyGlyGly by the CE-CL/absorption detection.
Conditions: PTFE capillary of 100 μm i.d., effective length of 70 cm for absorption detection and 90 cm for CL detection; applied voltage, 10 kV; running buffer, 10 mM carbonate buffer (pH 10.8) containing 4 μM microperoxidase; cell buffer, 10 mM carbonate buffer (pH 10.8) containing 400 mM hydrogen peroxide; detection, 280 nm for absorption and luminal system for CL detection; and sample, 50 μM ILITC-labeled compounds.

4. Conclusion

PTFE capillary is one of the most useful components in the various fields. However, PTFE capillary was less frequently introduced into the CE system. We newly developed the CE system incorporating the absorption/CL dual detection, taking advantage of PTFE capillary as a separation tube. ILITC labeled-biomolecules were analyzed by the CE system. The obtained data immediately indicated a unique CL performance in which the CL quantum yield of ILITC became extremely low under the present conditions due to the labeling procedure. The CE system will be applied to detect a trace amount of compound

possessing the CL property selectively among many compounds with the absorption property.

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References

1) R. Bakry, C.W. Huck, M. Najam-ul-Haq, M. Rainer, G. K. Bonn, “Recent advances in capillary electrophoresis for biomarker discovery”, *J. Sep. Sci.*, **30**, 192-201 (2007).
2) K. Tsukagosh, N. Jinno, R. Nakajima, “Development of a Micro-Total Analysis System Incorporating Chemiluminescence Detection and Application to Detection of Cancer Markers “, *Anal. Chem.*, **77**, 1684-1688 (2005).
3) J. Kawase, “Automated determination of cationic surfactants by flow injection analysis based on ion-pair extraction”, *Anal. Chem.*, **52**, 2124-2127 (1980).
4) T. Hara, K. Tsukagoshi, A. Arai, T. Iharada, “Determination of a Small Amount of Biological Constituent by Use of Chemiluminescence. XII. Highly Sensitive Immunoaffinity Chromatography”, *Bull. Chem. Soc. Jpn.*, **61**, 301-303 (1988).
5) M. Macka, W.C. Yang, P. Zakaria, A. Shitangkoon, E.F. Hilder, P. Andersson, P. Nesterenko, P.R. Haddad, “Poly(tetrafluoroethylene) separation capillaries for capillary electrophoresis. Properties and applications”, *J. Chromatogr. A*, **1039**, 193-199 (2004).
6) K. Tsukagoshi, S. Ishida, Y. Oda, K. Noda, R. Nakajima, “Compact Polytetrafluoroethylene Assembly-Type Capillary Electrophoresis with Chemiluminescence Detection”, *J. Chromatogr. A*, **1125**, 144-146 (2006).
7) J.Y. Zhao , J. Labbe, N.J. Dovichi, “Use of a sheath flow cuvette for chemiluminescence detection of isoluminol thiocarbamyl-amino acids separated by capillary electrophoresis”, *J. Microcol. Sep.*, **5**, 331-339 (1993).
8) M. Hashimoto, K. Tsukagoshi, R. Nakajima, K. Kondo, “Compact Detection Cell Using Optical Fiber for Sensitization and Simplification of Capillary Electrophoresis - Chemiluminescence Detector”, *J. Chromatogr. A*, **832**, 191-202 (1999).
9) K. Tsukagoshi, K. Sawanoi, M. Kamekawa, R. Nakajima, “Analytical Performance of Capillary Electrophoretic System with UV/CL or FL/CL Dual Detector”, *Chem. Lett.*, **32**, 894-895 (2003).
10) J.F. Rabek, J. Lucki, B. Raanby, Y. Watanabe, B.J. Qu, “Photoozonization of polypropylene. Oxidative reactions caused by ozone and atomic oxygen on polymer surfaces”, *ACS Symposium Series*, **364** (Chem. React. Polym.), 187-200 (1988).
11) K. Tsukagoshi, T. Nakamura, R. Nakajima, “Batch-type Chemiluminescence Detection Cell for Sensitization and Simplification of Capillary Electrophoresis”, *Anal. Chem.*, **74**, 4109-4116 (2002).