Capillary Electrophoresis with Chemiluminescence Detector Using On-capillary Detection

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We achieved capillary electrophoresis with chemiluminescence detector using on-capillary detection, taking advantage of the commercially available chemiluminescence reagent (LUMICA) and its oxidant. A mixture of dansyl tryptophan and dansyl glycine as a model sample migrated in two different running buffers, both containing LUMICA and the oxidant, indicating chemiluminescence was generated by the reaction of LUMICA, the oxidant, and dansyl α-amino acids. When the α-amino acids went through the detection window on the capillary, the chemiluminescence signals were observed with the chemiluminescence detector using on-capillary detection. This new concept of on-capillary chemiluminescence detection did not require any interface device between the electrophoresis capillary and the chemiluminescence detector.

Chemiluminescence (CL) has been used as an attractive detection technique in FIA, HPLC, and capillary electrophoresis (CE).[1–3] Recently, CL was recognized to match well with the concept of a micro-total analysis system (μ-TAS),[4] as no light source or spectroscope is required. In CE with CL detection, the CL reagent must be mixed with analytes eluted from the capillary at the outlet to produce CL.[5] Thus, all CL detections in CE are performed "end-capillary" (post column reaction), for which various kinds of devices that interface between the CE and CL detector have been developed.[6] The end-capillary detection sometimes requires complex detection cells, connectors, and tube-lines as well as expensive pumps for delivering the solutions, resulting in complicated analytical apparatus and procedures.

In the present study, we proposed for the first time to develop CE with a CL detector using "on-capillary" detection. In order to make on-capillary CL detection possible, we prepared a running buffer with LUMICA and its special oxidant, both of which are commercially available (LUMICA Corporation). LUMICA is reported to include oxalate CL reagent and a fluorescence compound (details are commercially sensitive and unable to be disclosed). The new concept of "on-capillary" detection using CL in CE is described below. When LUMICA is mixed with the oxidant, the mixture emits stable CL for several hours. The running buffer containing LUMICA and the oxidant showed stable background CL, which was used as base-line data for testing CE with CL detection. When a sample (fluorescence-labeled analyte) is injected into the capillary, the sample migrates toward the capillary outlet indicating CL in the running buffer. The CL from the sample is larger than the background CL, and the CL intensity is measured as a peak through the detection window on the capillary near the outlet.

As the mixture of LUMICA and the oxidant (volume ratio 1:1) showed very strong CL intensity, the mixture was diluted several hundreds times for CE with CL detection. When the mixture was diluted with an aqueous buffer, the CL intensity disappeared quickly. However, when the mixture was diluted with acetonitrile for use as a running buffer, the CE current was hardly detectable. Thus, we prepared two running buffers containing both LUMICA and the oxidant for CE with CL detection using on-capillary detection in the following two ways. Running buffer (A) contained acetonitrile, LUMICA, oxidant, and 10 mM phosphate buffer (pH 7.3) mixed in this order with volume ratios of 79:0.3:0.3:20, while Running buffer (B) contained acetonitrile, LUMICA, and oxidant mixed in this order with the volume ratios of 99:0.3:0.3, after which tetrabutylammonium perchlorate was dissolved in the solution to a final concentration of 5.0×10^{-3} M.

A capillary of 75-μm i.d. and 60-cm length (50-cm effective length) was used. The detection window (4 mm) on the capillary was produced by burning out the polyimmid-coating of the capillary surface. The capillary tube possessing the detection window was placed close on the center of the face of the photomultiplier tube. A mixture of dansyl tryptophan (Dns-Try) and dansyl glycine (Dns-Gly) was used as the model sample. The sample was prepared by dissolving α-amino acids with Running buffer (A) and Running buffer (B). The sample was injected by the gravity method (at 20-cm height and for 15 s). A voltage of 12 kV was applied between the ends of the capillary. The sample migrated toward the capillary outlet and the CL intensity was detected through the detection window by the photomultiplier tube.

We speculated that in spite of the high concentrations of LUMICA and the oxidant, a low background CL from a running buffer including LUMICA and the oxidant might be suitable for CE with CL detection using on-capillary detection. Although the fluorescence (FL) property does not always correspond to the CL performance, we examined the FL properties of 7 kinds of LUMICAs (VIOLET, PINK, ORANGE, GREEN, RED, WHITE, and YELLOW; all of them commercially available) by FL spectroscopy, in a preliminary experiment. We found that LUMICA (VIOLET) was most suitable for the present study because of its low dilution ratio and small FL intensity.

Figure 1 shows the electropherogram of the mixture of Dns-Try and Dns-Gly obtained with Running buffer (A). Stable background CL intensity was observed for at least 35 min and a 3–4-μA current was observed. The Dns-Try and Dns-Gly were separated and then detected with CL detector using on-capillary detection within 5 min. Figure 2 shows the electropherogram of the mixture of Dns-Try and Dns-Gly obtained with Running buffer (B). Stable background CL was observed, as was a 5–6-μA current. The Dns-Try and Dns-Gly were separated and detected within 10 min. The Dns-Try and Dns-Gly may be negatively

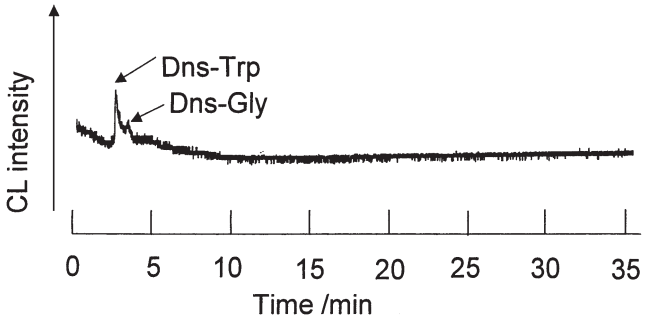

Figure 1. The electropherogram of a mixture of Dns-Try and Dns-Gly (1.0×10^{-4} M each). Running buffer (A).

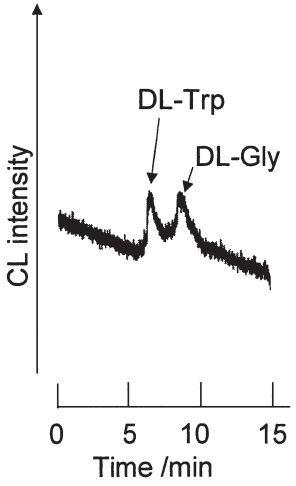

Figure 2. The electropherogram of a mixture of Dns-Try and Dns-Gly (1.0×10^{-3} M each). Running buffer (B).

charged owing to the dissociation of their carboxyl groups. The electrophoretic mobilities of Dns-Try and Dns-Gly turn to the capillary inlet because of these negative charges. The mobility of Dns-Try is smaller than that of Dns-Gly owing to their ratios of mass to charge. However, since the mobility of electroosmotic flow, which turns to the capillary outlet, was larger than that of electrophoresis, and thus the Dns-Try and Dns-Gly migrated to the capillary outlet in this order. Although the current in Running buffer (B) was larger than that in Running buffer (A), the components in the sample in Running buffer (A) migrated faster than those in Running buffer (B). The electroosmotic flow in Running buffer (B), which included mainly acetonitrile, might be small because of insufficient dissociation of silanol groups on the inner wall of the capillary.

The present CE with CL detector using on-capillary detection was developed on the basis of the new concept of CL analysis in CE, taking advantage of CL performance of LUMICA and its oxidant. Although the analytical conditions require optimization to improve selectivity and sensitivity, the concept of on-capillary CL detection does not require any devices to interface between the CE and CL detector, such as detection cells, connections, tube-lines, and high-performance pumps. Future research will focus on using LUMICA that does not contain fluorescence compounds in CE with CL detection, so as to improve sensitivity and selectivity in the near future.

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