α-Amino Acids Analysis by Capillary Electrophoresis with Chemiluminescence Detector Using Luminol–Hydrogen Peroxide–Cu(II) System

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(Received April 14, 2003; CL-030318)

Twenty kinds of α-amino acids were directly detected by capillary electrophoresis (CE) with a chemiluminescence (CL) detector using luminol–hydrogen peroxide–Cu(II) system. A batch-type CL detection cell to which a hydrogen peroxide solution was added was used. Samples migrated in a running buffer solution containing luminol and Cu(II), and eluted at a tip of a capillary to mix with the hydrogen peroxide. The catalytic activity of Cu(II) for luminol CL reaction increased in the presence of α-amino acids to induce CL peaks on the electropherograms. For example, aspartic acid was determined over the range of 1.1×10^{-7}–1.8×10^{-5} M with a detection limit of 0.6 fmol. A model mixture sample of glycine, aspartic acid, and glutamic acid was successfully separated and detected within 10 min.

Because of its simple optical system and low background nature, CL is expected to be an ideal detection method for CE. Recently, the feasibility of using CL detection in CE has been successfully demonstrated.[1–3] Several CL reactions, such as luminol, ruthenium (III) complex, and peroxyoxalate, have been utilized. As only a few substances show the native CL characteristic, derivatization of non-CL analytes prior to detection is generally required; however, derivatizing processes are often tedious and time-consuming. Quantitative labeling of a low-concentration analyte is also difficult.

Analysis of α-amino acids is very important in various fields including analytical chemistry, biochemistry, post genome chemistry, and medicine. α-Amino acids labeled with isoluminol and dansyl chloride were analyzed through CE with a CL detector.[1,2] In this study we found out that α-amino acids were directly detected in CE with a CL detector using a luminol–hydrogen peroxide–Cu(II) system without any labeling procedures. It is emerging as one of the most promising ways for the separation and determination of α-amino acids.

A batch-type detection cell was used for the present CE with a CL detector. The concept of the cell was originally proposed by us in the previous paper.[3] The detection cell was made of Teflon, which had 4-cm outer diameter, 2.5-cm height, and 8-mL inner volume. An optical fiber (a core diameter of 1.2 mm), a fused-silica capillary (50-µm i.d. and 50-cm length), and a platinum wire as a grounding electrode were fixed to the cell. That is, the cell also worked as an outlet reservoir including an electrolyte solution. The optical fiber was set up straight to the capillary with a distance of ca. 0.3 mm between them. A 10 mM phosphate buffer solution (pH 10.8) including 400 mM hydrogen peroxide was added into the cell. The CL light at the capillary outlet was captured by the optical fiber.

All of the reagents used were of commercially available grade. Ion-exchanged water was distilled for use. Luminol and all α-amino acids (L-isomers except for glycine) used were purchased from Nacalai Tesque. A 10 mM phosphate buffer solution (pH 10.8) was prepared. Sample solutions were prepared by dissolving α-amino acids with the phosphate buffer solution. A phosphate buffer solution (pH 10.8) containing 5.0×10^{-5} M luminol, 5.0×10^{-6} M Cu(II) (sulfate), and 5.0×10^{-5} M tartaric acid was used as a run-

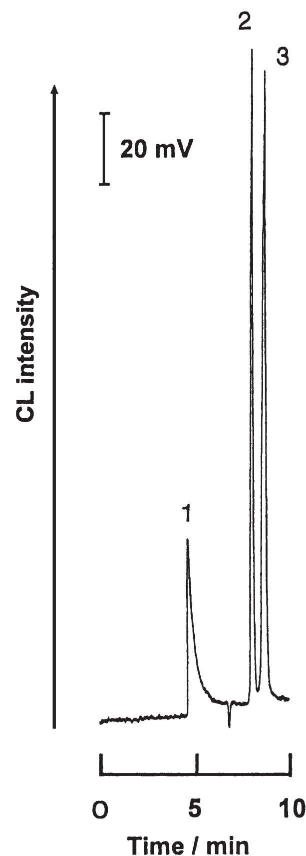

Figure 1. The electropherogram of the mixture sample solution of α-amino acids. 1, Glycine; 2, aspartic acid; and 3, glutamic acid. Conditions: Capillary, 50 cm length and 50 µm i.d. of fused silica; applied voltage, 12 kV; reagent, phosphate buffer (pH 10.8) containing 5.0×10^{-5} M luminol, 5.0×10^{-6} M Cu(II), and 5.0×10^{-5} M tartaric acid in the inlet reservoir and phosphate buffer (pH 10.8) containing 400 mM H_2O_2 in the outlet reservoir; and sample concentration, 1.0×10^{-5} M.

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ning buffer solution. A sample injection (ca. 5.6 nL) was performed through the use of gravity for 10 s at a height of 20 cm. A high voltage (12 kV) was applied to the electrodes using a DC power supplier. The sample migrated in the running buffer toward the CL detection cell and mixed with the reagents. The resulting CL at the capillary outlet was transported to the photosenser module through the optical fiber in the CL detector (Model EN-21, Kimoto Electric, Inc.). The output from the detector was fed to an integrator to produce electropherograms.

We examined twenty kinds of α-amino acids which were neutral α-amino acids (glycine, alanine, valine, leucine, isoleucine, asparagine, glutamine, serine, threonine, cysteine, methionine, phenylalanine, tyrosine, tryptophan, and proline), basic α-amino acids (lysine, arginine, and histidine), and acidic α-amino acids (aspartic acid and glutamic acid). All of them were directly detected by the present CE with the CL detector without any pretreatments such as a labeling procedure. The phosphate buffer solution containing a luminol and Cu(II) catalyst as a running buffer solution moved to the outlet from the inlet capillary through electroosmotic flow, and mixed with a hydrogen peroxide solution. Then, the CL intensity at the tip of capillary outlet was monitored as a base-line CL. When α-amino acids as a sample migrated in the capillary, mixing with the luminol and Cu(II) catalyst, and then emerged at the tip of the capillary to mix with the hydrogen peroxide, the CL intensity increased to make the positive peak. That is, the CL intensity of the luminol–hydrogen peroxide–Cu(II) system was magnified in the presence of α-amino acids. The Cu(II) must show a higher catalytic activity when it interacted with α-amino acids to form certain Cu(II)-α-amino acid complexes. It was also reported that the catalytic activity of Cu(II) for luminol-hydrogen peroxide CL reaction is at its maximum agitation when two of the four coordination sites are occupied by a ligand.[4,5] The increase of the catalytic activity of Cu(II) in the present study seems to be attributed to the formation of Cu(II)-α-amino acid complexes, that is, the occupation of two coordination sites by a α-amino acid.

Figure 1 shows the electropherogram of the model mixture sample solution of glycine, aspartic acid, and glutamic acid. They were successfully separated and detected. We examined the calibration curves of them. They were determined over a range of 2.0×10^{-7}-1.5×10^{-5} M with a detection limit of 1.1 fmol, 1.1×10^{-7}-1.8×10^{-5} M with 0.6 fmol, and 1.0×10^{-7}-1.8×10^{-5} M with 0.6 fmol, respectively. The correlation coefficients were 0.999. The CL intensity did not change during repeated injections at least up to 10 times; the relative standard deviation was 2.7%. We are now studying about the details of the experimental conditions and the possibility of expanding this method to other biological constituents such as peptides and proteins.

This work was supported by a Grant-in-Aid for Scientific Research (C) from the Ministry of Education, Culture, Sports, Science and Technology. The authors also acknowledge the financial support for this research by Doshisha University's Research Promotion Fund.

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Published on the web (Advance View) June 24, 2003; DOI 10.1246/cl.2003.634