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Short communication

Direct determination of amino acids and carbohydrates by high-performance capillary electrophoresis with refractometric detection

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Abstract

This is an initial report to propose a novel approach in high-performance capillary electrophoresis (HPCE) for the direct detection of compounds without natural absorbance in the UV and visible spectral range, such as amino acids and carbohydrates. A refractometry detector with the 2 nl cell (Applied Systems, Minsk, Belarus) was employed to identify amino acids and carbohydrates without derivatization. The first results are provided on separation of seven free amino acids in the phosphate running buffer and three free carbohydrates in the borate–sodium dodecyl sulfate running buffer and detection by refractometer. Fused capillaries of 50 or 75 μm internal diameter and separation voltage (10–23 kV) were applied. Detection limits ranged typically from 10 to 100 fmol and the response was linear over two orders of magnitude for most of the amino acids and carbohydrates. The HPCE system demonstrated good long-term stability and reproducibility with a relative standard deviation, less than 5% for the migration time ($n=10$). © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Amino acids and carbohydrates are important compounds in a wide range of samples, such as biological tissues and fluids, food and industrial products; their analysis is essential and often difficult, although they can be determined by a variety of analytical techniques. These compounds were traditionally separated by liquid chromatography; it took several years of intensive research to optimize the complete chromatographic separation of all the amino acids and the complex mixtures of carbohydrates. The analysis of amino acids is performed by pre- or post-column derivatization HPLC methods with some chromophore or fluorophore compounds and UV or fluorescence detection [1,2]. On the other hand, HPLC with pulsed-amperometric detection [3,4] or refractive index detection [5] serves as a common technique for underivatized carbohydrate analysis. For derivatized carbohydrates, HPLC with fluorescence [6,7], UV [8] detection or GC [9] was used. A great number of studies were reported on the HPCE separation of derivatized amino acids and carbohydrates, because of its high efficiency, low reagent consumption and rapid analysis. HPCE analysis of amino acids was mainly performed using

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derivatization methods with UV chromophore [10–12] or fluorophore [13–16] reagents. For HPCE carbohydrate analysis, various derivatization techniques [17–19] were utilized, because carbohydrates lacked both a charge and a strong chromophore in the UV range, although a couple of underivatized carbohydrate assays were also reported [20,21]. To obtain reliable data, chemical operations for tagging free amino acids and carbohydrates are disadvantageous. The separation of amino acids was described using capillary zone electrophoresis (CZE) with indirect detection at strong alkaline pH [22–24]. Indirect detection methods, which use the background absorber in the buffer system, broaden their application, but have some limitations of buffer contaminants. As mentioned above, the mixture of underivatized carbohydrates including acidic, neutral and amino sugars and sugar alcohols can be fully separated and determined using CZE with indirect detection at strong alkaline pH [25]. Although HPCE became a potent separation technique, a few practical methods for untagged amino acid [22,23,26,28–32] and carbohydrate [20–22,32] direct detections are available to date because of typically poor UV detection properties of these compounds.

In this paper we have tried refractive index detection for the direct determination of underivatized amino acids and carbohydrates. The first results on the separation of seven free amino acids by capillary zone electrophoresis and three free carbohydrates by micellar electrokinetic capillary chromatography (MECC) in fused capillaries with direct refractometry detection are shown.

2. Experimental

2.1. Chemicals and reagents

Amino acids and carbohydrates were from Sigma (St. Louis, MO, USA). All buffer components (sodium dibasic phosphate, sodium tetrahydroborate, sodium dodecyl sulfate and 3-(cyclohexylamino)-1-propanesulphonic acid) were purchased from Sigma. Sodium hydroxide was purchased from Merck (Frankfurt, Germany). Purified water from a Milli-Q system, Millipore (Molsheim, France) was used throughout the experiments. The liquids used for HPCE buffer systems were filtered through 0.22 μm membranes (Millipore) and degassed by vacuum for 10 min at 25°C. All solutions were mixed on a vibration mixer. The concentrations of amino acid working solutions ranged from 0.075 to 0.2 *M* and kept at −20°C.

2.2. Instrumentation

High-performance capillary electrophoresis analysis was performed on the HPCE system Cezar (Applied Systems, Minsk, Belarus). The electrophoregrams were recorded on a recorder Model 2210 (LKB, Sweden). For pH measurements a Digital pH Meter pH 525 (Wissenschaflich-Technische Werkstatten, Germany) was applied. The centrifuge Model CLN 12 (Nauchpribor, Lvov, Ukraine), microcentrifuge Eppendorf 1540 (Eppendorf, Germany), vibrating mixer IKA Vibro Fix (IKA, Germany) were used during sample preparation.

2.3. High-performance capillary electrophoresis

A running buffer of 75 m*M* sodium dibasic phosphate, pH 11 was used for amino acid separations. A running buffer of 25 mM sodium tetrahydroborate pH 8.0 was applied for carbohydrate identification. The separations were performed on uncoated fused-silica capillaries (Perkin-Elmer) of 90 cm (75 cm effective length)×50 μm I.D. Between injections, the capillary was conditioned for 5 min with 0.2 *M* NaOH and 5 min with running buffer. The separation voltage was +200 V/cm. The samples and standards were loaded on the capillary by hydrodynamic injection for 5–7 s.

3. Results and discussion

The refraction index detection was used in capillary electrophoresis earlier [30,31,33,34]. The purpose of this work was to apply the refraction index detection to direct HPCE analysis of free amino

acids and carbohydrates and to show by this analytical application that refractometry detection in HPCE is a very promising technique. There is a possibility of direct refractometry detection to identify these compounds in their mixtures. Alkaline conditions are provided for micellar electrokinetic capillary chromatographic separations with sodium dodecyl sulfate and capillary zonal electrophoretic separations, using zwitterionic buffers [3-(cyclohexylamino)-1-propanesulfonic acid (CAPS)], as well as strong electrolytes (borate and phosphate). Detection in the mentioned buffers had similar sensitivity results (data not shown). The best HPCE separation results ad interim are shown for amino acids in phosphate running buffer (Fig. 1) and carbohydrates in borate-sodium dodecyl running buffer (Fig. 2). The separation of seven amino acids was developed using capillary zonal electrophoresis as a separation technique; the three carbohydrates were separated by micellar electrokinetic chromatography. The concentration of each compound ranged from 0.075 to 0.2 *M*. The examined instrumental parameters included capillary diameter (50 and 75 μm I.D.) and separation voltage (10–23 kV). The given data (Figs. 1,2) was obtained using 50 μm fused capillaries and 18 kV (200 V/cm) voltage. The stabilizing temperature was achieved by different ways: detector cell thermostating, capillary thermostating by circulating

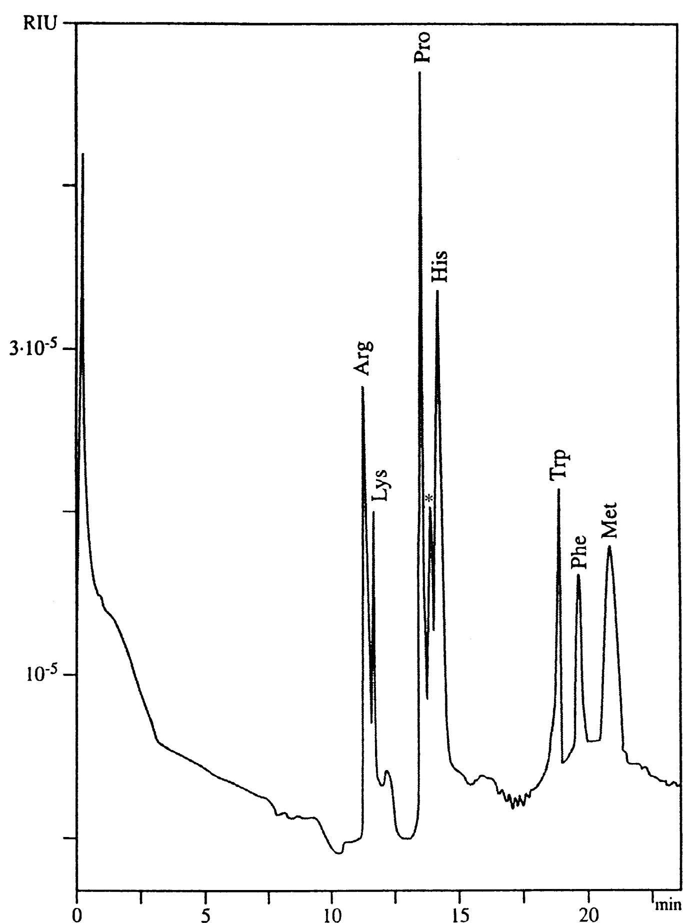

Fig. 1. HPCE identification of seven free amino acids with refractometry detection. Capillary: 90 cm (75 cm effective length)×50 μm I.D. Buffer: 75 m*M* phosphate, pH 11. The separation voltage: 200 V/cm. The peak marked by the asterisk corresponds to electroosmotic flow (EOF). Injection: electrokinetic, 7 s. Injected amounts: 1 ng for each compound.

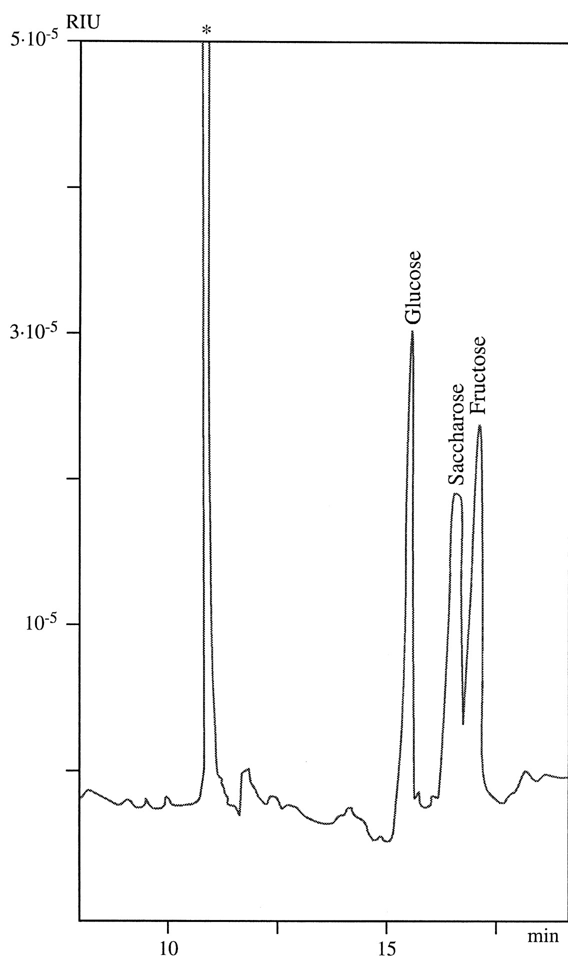

Fig. 2. HPCE identification of free carbohydrates with refractometry detection. Capillary: 90 cm (75 cm effective length)×50 μm I.D. The buffer: 25 m*M* borate, pH 8.0. The separation voltage: 200 V/cm. The peak marked by the asterisk corresponds to electroosmotic flow (EOF). Injection: electrokinetic, 5 s. Injected amounts: 300 pg for each compound.

liquid or isothermal air flow. The baseline drift caused by the Joule heating was minimal in case of simultaneous use of detector cell thermostating and capillary thermostating by circulating liquid (data not shown). But this drift was not dramatic even without any thermostabilizing. So, Figs. 1 and 2 demonstrate HPCE separation without special capillary or detector thermostating. The detector response, measured by the peak height, was linear over two orders of magnitude for the compounds tasted. Mass detection limits for amino acids and carbohydrates under the demonstrated conditions were typically 10–100 fmol. No obvious changes were observed either in the migration time or in the detector response during 10 successive runs (RSD<5%). This is well compared to electrochemical detection [26] and thermal lens indirect detection [27] in detection parameters and to the methods with chemical tagging in the sample preparation procedure.

4. Conclusion

Compared with the other techniques developed the HPCE method with refractive index detection has a number of advantages: (1) amino acids and carbohydrates, as the representatives of compounds which lack charge and/or a strong chromophore can be analyzed without derivatization, (2) sample preparation is minimum, (3) good working possibilities in various operating buffers and electrolytes.

Besides, it provides good reproducibility, linearity and appropriate sensitivity as well. These results indicate that the proposed detection type can be of use in HPCE techniques for research and further development of methodological approaches to routine analysis of amino acids, carbohydrates, and other non-chromophoric or fluorophoric compounds without chemical tagging procedures.

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