CATH.A NEURON CELL ANALYSIS ON A CHIP WITH MICELLAR ELECTROKINETIC CHROMATOGRAPHY

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Abstract

An on-chip electrophoresis analysis of the contents of CATH.a neuron cells is presented. A novel mixed-micelle MEKC separation was required to resolve twelve amino acids and neurotransmitters within five minutes.

Keywords: MEKC, neurotransmitter, amino acid, cell analysis

1. Introduction

Fast, robust methods for analyzing the chemical contents of neurons are necessary to determine the molecular basis of neuron structure and function. The CATH.a cell line is a model for differentiated CNS cells; CATH.a cells express catecholamines and other small amine neurotransmitters, high levels of tyrosine hydroxylase, neurofilaments, and synaptophysin[1]. We have developed methodology to probe the contents of CATH.a cells with separations on microfabricated glass devices.

Since the neurotransmitters of interest have similar electrophoretic mobilities, previous work in this field has employed long separation columns[2] with reduced electroosmotic flow (eof). To effect a fast separation on-chip with a relatively short separation column, Micellar Electrokinetic Chromatography (MEKC) with a unique mixed-micelle buffer was used.

2. Experimental

Separations were performed on glass microfluidic devices prepared using standard microfabrication and lithographic techniques. Analytes (standard solutions and homogenized cell contents) were derivatized with naphthalene-2,3-dicarboxaldehyde (NDA) prior to analysis. A variety of MEKC separation buffers were tried, including varying concentrations of detergents, organic modifiers, and background electrolytes.

Derivatized analytes were injected using pinched injection across a 100 μm double-T injector, and detected with LIF (405 nm Nichia diode laser). The signal was optically and spatially filtered and collected with a PMT, and plotted vs. time on a PC to form electropherograms. Peaks were identified by standard addition.

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3. Results and discussion

The best separations were performed in borate buffer (100 mM) with a mixed micelle detergent of SDS (80 mM) and the zwitterionic SB-12 (12 mM). Twelve neurotransmitters and amino acids were identified. As previously reported[1], dopamine and norepinephrine were detected, but their precursors (tyrosine and L-Dopa) were not (**Figure 1**). We speculate that future experiments using on-chip derivatization procedures may allow us to detect these precursors.

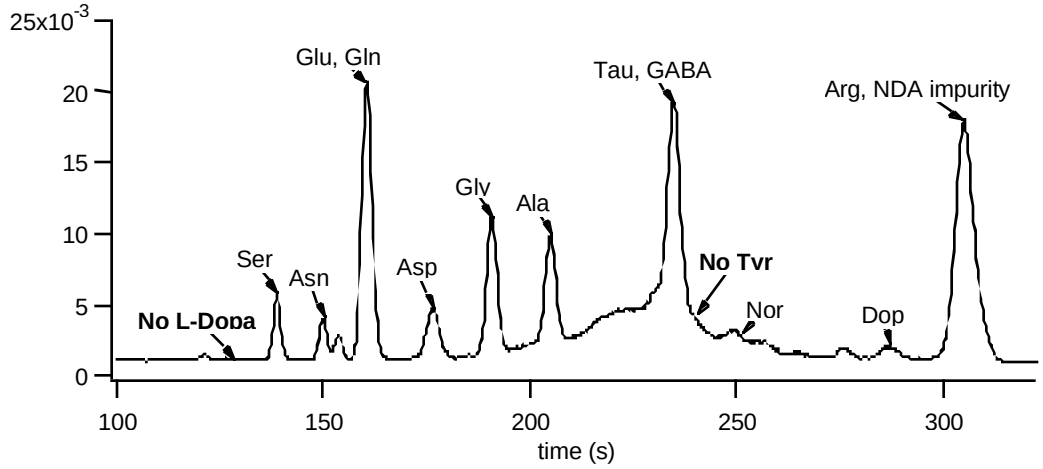

Figure 1: MEKC electropherogram of NDA-derivatized contents of CATH.a cells.

4. Conclusions

We have developed a robust, fast separation method for the analysis of small amine neurotransmitters in CATH.a neuron cells. We anticipate that this technique will be useful for neuroscientists investigating the biochemical basis of neuron structure and function.

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References

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