Chromatography
Separation Techniques

Wang, J Chromat Separation Techniq 2012, 3:2
DOI: 10.4172/2157-7064.1000e102

Editorial Open Access

Fluorophore – Labeled Substrate and CE for Monitoring Enzyme Activity

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Cancer is a disease of uncontrolled cell proliferation. This aberrant proliferative behavior is often caused by dysregulated signaling pathways, a major contribution comes from the abnormal enzyme activity. Development of anticancer drugs targeting these pathways has been a hot topic. Some reagents targeting the oncogenic signaling pathway, such as Phosphatidylinositol 3-kinase (PI3K), have reached clinic trials. In turn, a method of monitoring the enzyme activity is very valuable in the drug developing phase and in clinical applications on monitoring the reaction of patients during the treatment.

Chemical separation by CE with Laser Induced Fluorescence (LIF) detection has emerged as an effective means for studying a variety of biological analytes including oligonucleotides, amino acids, proteins, and lipid products because of high separation efficiency and an excellent detection limit. The Dovichi group coined the terms chemical cytometry and metabolic cytometry in 1999 [1] and has reported characterizing lipid metabolism by CE [2]. The Allbritton group demonstrated monitoring PI3K activity in single cell [3]. The basic idea of CE based enzyme activity detection is to deliver a substrate tagged with fluorescent dye into cells and the substrate can undergo biosynthesis or biodegradation by the corresponding enzymes. As long as the fluorescent tag remains intact, the metabolites can be monitored by CE-LIF.

Two main factors, fluorescent substrate and separation resolution of fluorescent substrate and products, need to be considered in developing CE based enzyme activity detection methodology. To detect the products of lipid metabolic enzymes by fluorescence-based techniques, a variety of fluorophore labeled lipid substrates have been developed, often with similar kinetics to the endogenous substrate. An ideal substrate should be cell permeable, otherwise complex methods such as delivering by carrier [4], micro injection or electroporation [5] need to be adopted for the delivery of cell-impermeant molecules. The separation resolution is also critical because the cell signaling pathways are extraordinarily complex. The substrate can be converted into more than one product in the cascade; some products often share a similar structure.

A trend of cancer treatment is the combination of reagents, for example combining DNA-damaging reagent and a multi-targeted protein kinase inhibitor [6]. For this reason, we can prospect that multiple channels (colors) analysis for monitoring two or more signaling pathways, called multiplex, will be highly desired in the future. The Dovichi group reported capillary electrophoresis with two-color fluorescence detection for the simultaneous study of two glycosphingolipid metabolic pathways [7]. To fulfill the requirement cancer treatment study, multiple fluorescent substrates of the tumor suppressive and oncogenic signaling pathways need to be designed and synthesized. Although one opinion states that the resolution of chemical separation is high enough to differentiate the changes of substrates and products of the cell signaling pathways, multiplex analysis will definitely provide us more information on the cell signaling process, especially the cross-talk of different cell signaling pathways.

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Received April 28, 2012; **Accepted** April 30, 2012; **Published** May 02, 2012

Citation: Wang K (2012) Fluorophore–Labeled Substrate and CE for Monitoring Enzyme Activity. J Chromat Separation Techniq 3:e102. doi:10.4172/2157-7064.1000e102