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Metabolome analysis based on capillary electrophoresis-mass spectrometry

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ABSTRACT

Capillary electrophoresis-mass spectrometry (CE-MS) has emerged as a powerful new tool for comprehensive analysis of charged compounds. In this review, we provide a general description of the application of CE-MS in metabolome analysis, including the separation modes of CE, various interfaces and the mass spectrometers used. We also discuss strategies for sample pretreatment, data processing and peak identification, which are important processes in metabolome analysis. In addition, we highlight a number of new techniques to improve metabolite extraction, peak resolution and sensitivity. Finally, we provide some general conclusions and future perspectives.

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1. Introduction

Metabolomics, or metabolome analysis, is a rapidly growing research field for comprehensive measurement of metabolites (typically <1.5 kDa) in a biological sample. The metabolome, together with the proteome, peptidome, elementome and transcriptome, provides information on cellular function and defines the phenotype of a cell in response to genetic or environmental changes [1]. Most metabolites are not organism specific, and, for humans, there are more than 40,000 metabolites registered in the latest Human Metabolome Database (HMDB) [2].

However, because of wide variations in the chemical and physical properties and concentrations (pmol to mmol) of the metabolites, analysis of all metabolites is challenging [3]. Variations in the extraction efficiencies for different classes of metabolites also complicate absolute quantification in metabolomics. For these reasons, metabolomics has been performed with different analytical platforms combined with selective metabolite-extraction procedures for metabolites with similar chemical properties.

Currently, metabolomics is performed using nuclear magnetic resonance (NMR) spectroscopy, or chromatographic or electrophoretic separation methods combined with mass spectrometry (MS), such as gas chromatography (GC)-MS, liquid chromatography (LC)-MS and capillary electrophoresis (CE)-MS.

Abbreviations: BGE, Background electrolyte; CE, Capillary electrophoresis; CEC, Capillary electrochromatography; EOF, Electro-osmotic flow; ESI, Electrospray ionization; FT-ICR, Fourier transform ion cyclotron resonance; GC, Gas chromatography; HMDB, Human metabolome database; IT, Ion trap; LC, Liquid chromatography; MS, Mass spectrometry; NMR, Nuclear magnetic resonance; Q-TOF, Quadrupole time-of-flight; SPE, Solid-phase extraction; TOF, Time-of-flight.

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GC-MS has been used extensively in metabolomics because of its high resolution, selectivity and sensitivity. It is a relatively simple, inexpensive method. The advantage of GC-MS compared with other techniques is that universal electron-impact ionization provides responses for all metabolites and characteristic, reproducible and standardized mass-spectral fingerprints [4]. Peaks in mass spectra can be identified by comparison to those in public and commercial databases. However, it is somewhat limited by the need for derivatization procedures for each metabolite. Derivatization is generally time-consuming, increases the complexity of sample preparation, and increases variance in the analysis [1]. Moreover, even after derivatization, a considerable number of metabolites are still non-volatile, so they cannot be analyzed by GC-MS.

LC-MS can be used for analysis of a wide range of compounds without derivatization [5]. Many LC methods have been developed for various metabolites of interest. Reversed-phase LC (RPLC) using C-18 columns is widely used for global profiling of metabolites. Ion-pair RPLC-MS [6] and hydrophilic interaction chromatography-MS [7] are commonly used to analyze polar metabolites. A critical aspect in quantitative analysis using LC-MS is matrix effects, which can lead to significant differences in the response of an analyte in a sample compared with the response in a pure standard solution [8].

CE-MS has emerged as a powerful new tool for the analysis of charged compounds. In contrast to GC and LC, which utilize the difference in interaction between the analyte and the stationary phase, CE achieve separation based on differences in the mass-to-charge ratios (*m/z*), and neutral compounds are also separated from ions [9]. Metabolites are first separated by CE and selectively detected using MS by monitoring ions over a range of *m/z* values. Many metabolites, especially those in the central carbon metabolism, possess amino, hydroxyl, carboxyl, and phosphate groups and are charged, making them suitable for CE-MS analysis.

NMR spectroscopy is advantageous because it is non-destructive and has high throughput, and sample preparation is simple [10]. These advantages are apparent in widespread use of proton-NMR metabolome analysis. However, it generally has low sensitivity and can only detect fewer than 100 metabolites.

Many reviews have been published that highlight the technical developments and various applications of CE-MS-based metabolomics [3,9–13]. This review provides a general description of the application of CE-MS in metabolomics, including the CE-separation modes, interfaces and mass spectrometers used. We also describe strategies for sample pretreatment, data processing and peak identification. In addition, we detail a number of new techniques for improving metabolite extraction, peak resolution and sensitivity. These techniques include electro-driven sample preparation, the use of Orbitrap MS and low sheath flow/sheathless interfaces. Finally, we provide some general conclusions and future perspectives.

2. CE-MS for metabolome analysis

2.1. Separation mode

Fig. 1 shows the most frequently used CE-MS set-ups for metabolomics. For cationic metabolites, separations are carried out on a fused-silica capillary, the capillary inlet is set as the anode and the MS is coupled to the cathode (Fig. 1A). To analyze as many cations as possible simultaneously, a low pH solution (concentrated formic acid or acetic acid) is employed as the background electrolyte (BGE) to ensure all metabolites carry positive charges [14]. In this manner, amino acids, amines, nucleobases, nucleosides and small peptides can be efficiently separated by CE and then selectively and sensitively detected by MS.

Because the metabolites of key pathways for cellular energy production, such as the glycolysis, pentose-phosphate and tricarboxylic-acid pathways, are almost entirely anionic species, anionic metabolome analysis is important. Two methods are commonly used for anionic analyses.

The first uses a BGE with a high pH (above 7.5) to create strong electro-osmotic flow (EOF). The electrophoretic polarity configuration for cationic metabolome analysis (Fig. 1B) is also used for this anionic analysis. In this setting, all anionic metabolites migrate toward the anode. Concurrently, strong EOF movement occurs in the opposite direction (from anode to cathode), and most of the

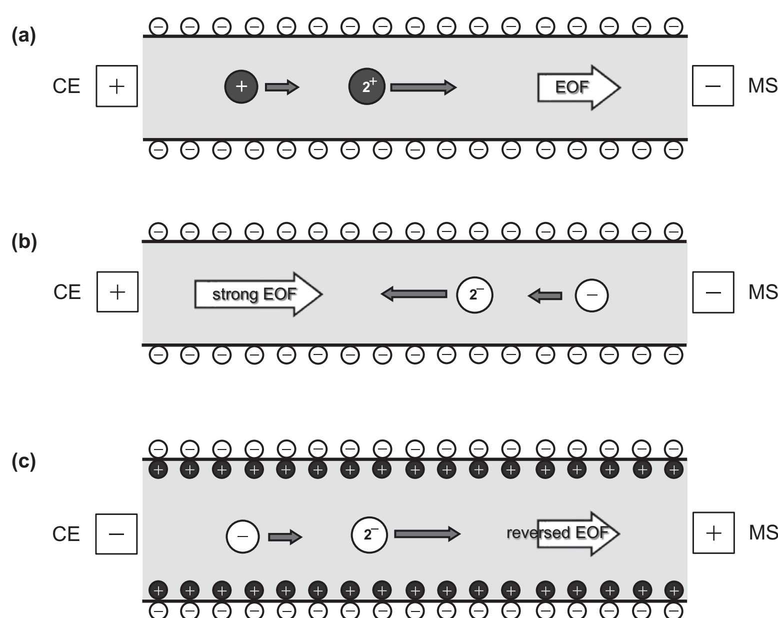

Fig. 1. CE-MS metabolome analysis: (a) cationic metabolome analysis; (b) anionic metabolome analysis with normal EOF; (c) anionic metabolome analysis with reversed EOF.

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