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Multidimensional capillary array liquid chromatography and matrix-assisted laser desorption/ionization tandem mass spectrometry for high-throughput proteomic analysis

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Abstract

A two-dimensional capillary array liquid chromatography system coupled with matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS) was developed for high-throughput comprehensive proteomic analysis, in which one strong cation-exchange (SCX) capillary chromatographic column was used as the first separation dimension and 10 parallel reversed-phase liquid chromatographic (RPLC) capillary columns were used as the second separation dimension. A novel multi-channel interface was designed and fabricated for on-line coupling of the SCX to RPLC column array system. Besides the high resolution based on the combination of SCX and RPLC separation, the developed new system provided the most rapid two-dimensional liquid chromatography (2D-LC) separation. Ten three-way micro-splitter valves used as stop-and-flow switches in transferring SCX fractions onto RPLC columns. In addition, the three-way valves also acted as mixing chambers of RPLC effluent with matrix. The system enables on-line mixing of the LC array effluents with matrix solution during the elution and directly depositing the analyte/matrix mixtures on MALDI plates from the tenplexed channels in parallel through an array of capillary tips. With the novel system, thousands of peptides were well separated and deposited on MALDI plates only in 150 min for a complex proteome sample. Compared with common 2D-LC system, the parallel 2D-LC system showed about 10-times faster analytical procedure. In combination with a high throughput tandem time of flight mass spectrometry, the system was proven to be very effective for proteome analysis by analyzing a complicated sample, soluble proteins extracted from a liver cancer tissue, in which over 1202 proteins were identified.

Keywords: Multidimensional liquid chromatography; Array; Proteomic analysis

1. Introduction

Proteomics, the systematic study of all proteins expressed in cells, tissues or organisms, has placed tremendous demand for highly efficient analytical platforms for protein profiling of complex biological samples. Current proteomics is mostly based on two-dimensional gel electrophoresis (2D-GE) separations of proteins followed by mass spectrometric analysis [1–4]. 2D-GE is a powerful separation technique, which fulfils the two-dimensional orthogonal separation of proteins by isoelectric point and molecular weight, and allows the separation of thousands of proteins in a single analysis. However, the technical limitations associated with 2D-GE, such as labor-intensive, relatively low throughput and difficulties in identifying low-abundance, more basic and large proteins, impeded thorough proteome characterization [5].

Chromatography is a highly efficient separation technique and can resolve most problems met in gel-based proteomic analyses. In recent years, many multidimensional analysis platforms based on liquid chromatography (LC) or capillary electrophoresis (CE) were investigated, including LC–LC [6–12], LC–CE/CE–LC [13–16], and CE–CE [17–19].

Mass spectrometry (MS) is playing a prominent role for protein identification, as a result of the development of two new ionization methods, electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI). Based on direct LC separation of proteolytic digests of proteins and tandem

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mass spectrometry (MS/MS) analysis of peptides, shotgun proteomics approach has been proved to be a powerful tool to proteome profiling. Using 2D-LC coupled to ESI MS/MS, Yates' group pioneered an automated technique for shotgun proteomics, termed multidimensional protein identification technology (MUDPIT) [20]. In MUDPIT, a biphasic column packed with two different types of packings (SCX and RPLC particles) was used for 2D-LC separation of protein digests, and ESI MS/MS was used for the proteins identification. By 15 SCX and RPLC elution cycles for each run of each fraction, in 83 h for three fractions, thousands of proteins were identified in the *S. cerevisiae* proteome [21].

Because ESI-MS can be directly coupled to LC, ESI-MS in conjunction with 2D-LC is increasingly applied for proteomics research [12,16,20–23]. For the relatively simple proteome, such as microbial and lower eukaryotic proteomes, the protein identification capacity of 2D-LC-ESI/MS is sufficient for the direct achievement of the comprehensive proteins characterization from the proteins mixture. However, for the extreme complex proteome, such as mammalian proteome, considerable co-elution of peptides in 2D-LC, ion suppression and limited spectral recording duty cycle of MS may yield an incomplete analysis. The approach to repetitive 2D-LC/ESI-MS analyses for one sample was taken to increase the coverage of proteome and achieve more comprehensive analysis [24], which led to increased sample consumption, and the analysis throughput further reduced.

MALDI [25,26] has shown to be complementary to ESI in producing biomolecular ion for MS analysis [27]. In addition, MALDI–MS offers higher tolerance toward sample contaminants such as buffers, salts, and surfactants. In recent years, many coupling approaches for LC and MALDI have been reported [28–32]. By deposition of LC effluents as discrete spots or track on a plate, LC separation can be easily combined with MALDI–MS. For the offline coupling of LC separation to MALDI–MS, LC-MALDI-based shortgun approach enables more MS/MS analyses at a given point in a separation because the extended period can be devoted to the analysis, which facilitates improved proteome coverage. However, the high-efficiency separations of complex sample prior to MALDI analysis achieved by the current 2D-LC are usually performed first by SCX fractionation followed by sequential RPLC elutions in the second dimension. The approach is time-consuming, and its throughput is significantly limited by running LC one by one [12,20–23].

The advantage of the multiple-column LC system recently became increasingly attractive in single-dimension separation [33–38]. To increase the throughput for multidimensional LC analysis, an online 2D array LC system using 10 capillary columns in parallel as the second separation dimension was first developed in this work. The 2D-LC array system allows the concurrent gradient elution of 10 fractions displaced in the first separation dimension, and therefore, the total chromatographic separation time for a proteomic sample was reduced by ca. 10-fold. In addition, the system enables on-line mixing of the effluents of tenplexed LC with matrix and depositing of the analyte/matrix mixtures from 10 channels in parallel by one spotting system. With MALDI-time-of-flight (TOF)-TOF-MS, the performance of the system for high-throughput and high-resolution proteome analysis was demonstrated using the soluble fraction of D20 liver cancer tissue extracts.

2. Experimental

2.1. Materials and reagents

Fused-silica capillaries (50 μm I.D., 375 μm O.D.; 75 μm I.D., 375 μm O.D.; 250 μm I.D., 375 μm O.D.; and 320 μm I.D., 450 μm O.D.) were from Yongnian Optical Fiber Factory (Yongnian, Hebei, China). Packing materials, 5 μm spherical silica gel Zorbax BP-Sil and 5 μm Zorbax 300 SB-C8 were obtained from DuPont (Wilmington, DE, USA), and Agilent Technologies (Palo Alto, CA, USA), respectively. Poros SCX packing was kindly supplied by PerSeptive Biosystems (Framingham, MA, USA). HPLC-grade acetonitrile (ACN) and trifluoroacetic acid (TFA) were from Merck (Darmstadt, Germany). HPLC grade ammonium acetate ($CH_3CO_2NH_4$) was from Tedia (USA). MALDI matrix a-cyano-4-hydroxycinnamic acid (CHCA) was from Aldrich (Milwaukee, WI, USA). All protein standards and sequencing grade trypsin were obtained from Sigma.

2.2. Preparation of samples

Cytochrome *c* was dissolved in 100 mmol/L NH_4HCO_3 buffer at a concentration of 3 mg/mL and denatured by boiling for 15 min. The protein solution was digested overnight at 37 °C with sequence grade trypsin at a ratio of 25:1 (w/w).

The liver cancer tissue of D20, a human hepatocellular carcinoma model in nude mice with high metastatic potential [39,40], was obtained from Liver Cancer Institute in Zhongshan Hospital, Fudan University. D20 liver cancer tissue was cleaned with Milli-Q water to remove contaminants, cut into small pieces, and homogenized in 1 mmol/L phenylmethanesulfonyl fluoride (PMSF) aqueous solution using glass homogenization vessel in ice bath. The resulting homogenate was swirled for 20 min and centrifuged for 10 min at 12,000 × *g*. The supernatant was collected, fractionated in aliquots and stored at −20 °C till further analysis. The protein concentration was measured by a Bio-Rad assay using bovine serum albumin (BSA) as standard. The solution containing 1 mg of proteins was reduced and denatured by boiling at 100 °C for 15 min, and then was digested overnight at 37 °C with sequence grade trypsin at a ratio of 50:1 (w/w). The digests were acidified by adding TFA before analyses.

2.3. Two-dimensional capillary array liquid chromatography system

A diagram of the automated two-dimensional capillary array liquid chromatography system is shown in Fig. 1. The 2D-LC system consists of two Agilent 1100 series capillary pumping systems (Agilent Technologies), a Famos micro-autosampler (LC Packings, Dionex), an SCX column, a 10 capillary RP columns, a multi-channel interface, 10 thermo expansion pumps [41] (designed and constructed in our laboratory), and a Probot