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Peptide enrichment by microfluidic electrocapture for online analysis by electrospray mass spectrometry

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Abstract

Identification of peptides from a complex mixture can be difficult because of the wide concentration range and the different ionization efficiencies of peptides during analysis by electrospray ionization (ESI) mass spectrometry (MS). Preconcentration methods are necessary to allow low-abundance and low-intensity peptides to reach the ionization threshold of the mass spectrometer. Here we demonstrate peptide enrichment based on electroimmobilization. Peptides are immobilized without the use of solid support or chemical binding by application of an electric field along a microflow stream in an electrocapture cell. Once enriched/preconcentrated inside the cell, they are released by removal of the electric field and via an interface with an electrospray emitter are submitted to online mass spectrometric analysis. Tandem mass spectrometric analysis of a peptide mixture containing hemoglobin, myoglobin, bovine serum albumin (BSA), and cytochrome *c* was successful. Amplification factors up to 16-fold were achieved with improvement of the signal-to-noise values for the preconcentrated sample. The limit of detection for one of the preconcentrated peptides was 3.6 fmol.

Keywords: Peptide enrichment; Preconcentration; Microfluidic electrocapture; Electrospray ionization (ESI) mass spectrometry (MS); Undersampling; Ionization efficiency; Peptide identification; Signal-to-noise (S/N) ratio; Limit of detection (LOD)

A main effort in proteomics is identification of complex biological mixtures using two-dimensional gel electrophoresis (2-DE)[1] integrated with electrospray ionization (ESI) or matrix-assisted laser desorption ionization (MALDI) mass spectrometry (MS). Despite advances in these technologies, the dynamic range of proteomes and the large spread in their protein amounts, prefractionation and concentration are required for successful analysis [1]. Low-abundance proteins (present in amounts six orders less than the most abundant proteins) could still have crucial biological significance [2]. Also, other molecules present at low concentration in blood, such as cytokines and hormones, polypeptides with posttranslational modifications, protein isoforms, and small charged molecules, demand selective concentration and enrichment methods to allow detection, characterization, and quantification [3–5]. Gel-free approaches for sample enrichment and subsequent detection have emerged, employing different types of affinity columns that trap and concentrate the analyte coupled to liquid chromatography (LC) followed by mass spectrometric analysis [6,7]. Examples are C18 columns for analyte trapping and enrichment [8] and trypsin-modified C4 columns for combined protein preconcentration and digestion [9]. Both are integrated into LC systems that, in turn, are coupled to mass spectrometers. Other enrichment methods described involve titanium dioxide columns for phosphopeptides [10], interaction of peptides with specific antibodies immobilized on magnetic beads [11], and reduction of the concentration dynamic range via solid-phase ligand

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[1] *Abbreviations used:* 2-DE, two-dimensional gel electrophoresis; ESI, electrospray ionization; MALDI, matrix-assisted laser desorption ionization; MS, mass spectrometry; LC, liquid chromatography; BSA, bovine serum albumin; CE, capillary electrophoresis; ITP, isotachophoresis; MS/MS, tandem mass spectrometry; DTT, dithiothreitol; IAA, iodoacetamide; LOD, limit of detection; FWHM, full-width half-maximum; DDA, data-dependent acquisition; PLGS 2.2.5, ProteinLynx Global SERVER 2.2.5; S/N, signal-to-noise; LC–MS, liquid chromatography–mass spectrometry; TIC, total ion current.

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libraries [12,13]. Most of these efforts require preparation steps, such as packing, equilibration, and binding, with accompanying possible losses, especially with low-concentration samples.

Microfluidic systems minimize sample handling via integration of key applications such as cleanup, preconcentration, separation, and analysis [14–17]. Often, large-scale designs are redesigned to small-scale systems and/or microfluidic chips. This implies that traditional elements such as column packing materials and affinity beads, elements that could compromise analysis speed and sensitivity, are still present [18,19]. A chip containing an HPLC column, a sample enrichment column, and a nanoelectrospray tip has been described [20]. The emphasis of that design was to reduce dead volumes between the components and to minimize signals due to chemical background that can exceed the highest peak in a mass spectrum, a concern when working with very low concentrations. Peptides from a 600-amol bovine serum albumin (BSA) sample were identified. A microfluidic method that does not require solid support is capillary electrophoresis (CE) [21,22]. Over the past 20 years, several successful interface designs between this technique and ESI–MS have been presented [23–28]. A further approach is the combination of isotachophoresis (ITP) with CE before mass spectrometric detection [29,30]. In a first step, sample stacking is performed via ITP, followed by separation using CE, a combination particularly useful for preconcentration/enrichment of trace analytes [29,30].

The microfluidic electrocapture technique is free of solid support, holding the prospect of facilitating several proteomic sample preparations. Molecules are immobilized by application of an electric field in a microflow stream. This makes it possible to clean, concentrate, and separate analytes while keeping sample manipulation low. Successful sample preparation with this technique has been demonstrated in combination with MALDI– and ESI–MS [31,32]. The purpose of the current investigation was to demonstrate enrichment for peptide mixtures with the microfluidic electrocapture technology for subsequent online analysis of the preconcentrated samples by ESI–MS and ESI tandem mass spectrometry (MS/MS).

Materials and methods

Proteins and chemicals

Myoglobin, hemoglobin, BSA, cytochrome *c,* dithiothreitol (DTT), iodoacetamide (IAA), and ammonium formate were obtained from Sigma (St. Louis, MO, USA). Methanol was obtained from Rathburn Chemicals (Walkerburn, Scotland). Porcine sequencing-grade modified trypsin was obtained from Promega (Madison, WI, USA), and water from a Milli-Q purification system (Millipore, Billerica, MA, USA).

Trypsin digestion

Stock solutions were prepared at concentrations of 5 mg/ml for each of the four proteins, which were then digested separately. Protein stocks (12.5 μl) were diluted 1:1 with water. Carbamidomethylation was carried out by incubation with 5 μl of 45 mM DTT at 50 °C for 15 min, after which 5 μl of 100 mM IAA was added, and the mixture was left at room temperature for 15 min. Following alkylation, reagents were diluted by the addition of ammonium bicarbonate (64 μl, 7.5 mM, pH 8.0), and 1 μl trypsin (1μg/μl in 50 mM acetic acid) was added for digestion overnight at 37 °C. Resulting digests were stored at –20 °C. For experiments, a mixture of 5.3 μl cytochrome *c* digest, 3.3 μl BSA digest, 2.0 μl myoglobin digest, and 1.5 μl hemoglobin digest was added to 19.9 μl of 10 mM ammonium formate (pH 8.7) and 30% methanol to a final volume of 32 μl. For peptide identifications and limit of detection (LOD) determinations, the samples were diluted with 10 mM ammonium formate (pH 8.7) and 30% methanol as indicated below.

Electrocapture cell

The microfluidic device was manufactured as follows. On a piece of PEEK tubing (127 μm i.d. and 512 μm o.d., Upchurch, Oak Harbor, WA, USA), two small openings were made with a scalpel and covered with a conductive tubular cation-selective membrane of poly(tetrafluoroethylenesulfonate) material (PermaPure, Toms River, NY, USA) with an inner diameter of 330 μm and an outer diameter of 610 μm. The membrane-covered openings separated by 2 cm constitute the electric junctions, and each was placed into a separate electrode chamber made from 500 μl Eppendorf tubes (Eppendorf, Hamburg, Germany).

Electrocapture system

Before use, the electrocapture cell was placed into an electrocapture instrument (Biomotif, Danderyd, Sweden). The main components were a pump with a 250-μl syringe, a microinjector, a power supply, and a holder keeping the cell and electrodes of platinum wire in place. Software allowed control of the pump and power supply and also monitored voltage and current during each run. The instrument was operated with the anode located at the upstream electric junction.

The electrode chambers were filled with 100 mM ammonium formate (pH 8.7), and the syringe was filled with 10 mM ammonium formate (pH 8.7) and 30% methanol. The sample was injected into the system via a 6-μl loop connected to the microinjector. The setup is shown in Fig. 1.

Mass spectrometry

The electrocapture cell was connected to a Q-TOF Ultima API mass spectrometer (Waters, Milford, MA,