Available online at www.sciencedirect.com
ScienceDirect
Analytica Chimica Acta 597 (2007) 41–49
ANALYTICA CHIMICA ACTA
www.elsevier.com/locate/aca

Off-line coupling of preparative capillary zone electrophoresis with microwave-assisted acid hydrolysis and matrix-assisted laser desorption ionization mass spectrometry for protein sequencing

Mahmoud M. Yassine, Nan Guo, Hongying Zhong, Liang Li, Charles A. Lucy *

Department of Chemistry, Gunning/Lemieux Chemistry Centre, University of Alberta, Edmonton, Alberta, Canada T6G 2G2

Received 22 February 2007; received in revised form 25 May 2007; accepted 31 May 2007
Available online 3 June 2007

Abstract

An off-line coupling of capillary electrophoresis (CE) with microwave-assisted acid hydrolysis/matrix-assisted laser desorption ionization mass spectrometry (MAAH/MALDI) has been developed for protein identification and characterization. Preparative scale protein separations enable collection of 10–50 pmol of purified cytochrome *c* for subsequent sequencing using MAAH/MALDI. To reduce protein adsorption onto the silica surface, the cationic surfactant-based coatings, dimethylditetradecylammonium bromide and dimethyldioctadecylammonium bromide, are employed. The choice of the buffer conditions is critical for both the preparative CE and MAAH/MALDI method. The use of high buffer concentrations (100 mM Bis–tris) reduces electromigration dispersion, but suppressed MALDI ionization such that a peptide sequence coverage of only 80% was achieved at a sample loading of 40 g L^{-1} of each cytochrome *c*. By reducing the buffer concentration to 25 mM Bis–tris, the sequence coverage increased to 95% at a sample loading of 40 g L^{-1}.

Keywords: Capillary electrophoresis; Microwave; Protein digestion; Preparative separation; Proteomics; Matrix-assisted laser desorption ionization time-of-flight mass spectrometry

1. Introduction

Two-dimensional gel electrophoresis (2D-gel) followed by mass spectrometric identification of proteolytic digestions is the workhorse in proteomics [1–4]. 2D-gel electrophoresis offers an impressive resolving power for complex protein mixtures by arraying them based on their isoelectic point and size. However, this technique is time-consuming, labor-intensive, difficult-to-automate and has low reproducibility [5]. Extraction of proteins from the gel for subsequent characterization is not straightforward, requiring special methods that increase the analysis time. For instance, passive elution requires 4–8 h to extract protein from a gel [6], while micro-electroelution requires at least 2 h [7]. Additionally, all methods of extracting protein from 2D-gels exhibit sample loss [2]. More importantly, sodium dodecyl sulfate (SDS; ∼2%) binds extensively to proteins [8–10]. Thus, it is not possible to extract the whole protein from the gel in its native form [11], and the extracted sample must be cleaned to remove or minimize the amount of SDS bound to the isolated protein. The presence of the even small amounts (<0.05%) of SDS significantly degrades both the signal-to-noise ratio and resolution of mass spectroscopy (MS) [12–14]. Finally, enzymatic digestion of the protein requires at least 12 h before the resultant peptide mixture can be analyzed by MS [15,16].

Extensive efforts have been devoted to the development of non-gel-based techniques to facilitate isolation of preparative quantities of proteins. One such technique is capillary isoelectric focusing (CIEF) [17,18], in which 0.3 μg (∼25 pmol) of each protein have been isolated using100 μm capillaries [19]. However, the IEF ampholytes must be removed before any further protein characterization can be performed. In addition, the mobilization step can cause deterioration of the pH gradient created in the column during the focusing step in CIEF. This results in poor reproducibility, longer analysis times and degradation of resolution between the focused zones [18]. Furthermore, proteins with very comparable isoelectric points cannot be separated at preparative scales using CIEF (estimated resolution is 0.05 pH units [19]). Thus, the separation of bovine and horse cytochrome

* Corresponding author. Tel.: +1 780 492 0315; fax: +1 780 492 8231.
E-mail address: charles.lucy@ualberta.ca (C.A. Lucy).

0003-2670/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.aca.2007.05.054

c, whose theoretical p*I*s differ by only 0.07 [20], would be challenging by CIEF.

Capillary zone electrophoresis (CZE) has potential as a preparative method for proteomics. CZE is performed in simple buffer solutions. Thus, it is possible to purify and collect proteins from a mixture without altering their activity. However, isolation of microscale amounts of separated protein components is hampered by CZE's small format. Typically, less than 100 fmol of protein is injected onto a 50 μm i.d. capillary [21]. Multiple sequential fraction collections can be used to increase the amount of collected analyte [22]. However, this approach is time-consuming and impractical.

Recently, Schoenherr et al. reported a proof-of-principle for a fully automated CE-Microreactor-CE-MS/MS for bottom-up protein characterization [32]. In this method, proteins are first separated by poly(vinyl alcohol) (PVA) coated CE to reduce protein adsorption. The separated protein fractions are digested with an on-line microreactor incorporated at the end of the capillary. The resultant peptide mixture formed in the reactor is then separated by a second PVA-coated CE and characterized by MS. The sequence coverage for cytochrome *c* and myoglobin were 48% and 22%, respectively.

The introduction of matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) [23,24] has provided a powerful approach for the analysis of small quantities of protein and expanded the utility of mass spectrometry to proteomic applications. For instance, mass determination for proteins by MALDI-MS requires less than 100 fmol of purified protein. Direct deposition of the CE eluent onto a MALDI target yields detection limits of 0.25 fmol [25,26], while collection of CE fractions and then performing off-line MALDI-MS analysis yields a 1.2 fmol LOD [21,27,28]. An additional advantage of fraction collection is that the material collected can be used for further protein characterization. Compared to electrospray ionization (ESI) which is very powerful for on-line detection of peptides and proteins separated by a solution-based separation technique such as LC or CE, MALDI shows greater tolerance to salts, surfactants, and other impurities in protein or peptide samples. In addition, MALDI shows less ion suppression than ESI for peptide and protein mixture analysis. Thus, MALDI is a preferred technique for direct analysis of peptide and protein mixtures.

Microwave-assisted acid hydrolysis (MAAH) is a simple and rapid method for generating polypeptide ladders for peptide sequencing of a protein and for characterization of protein modifications [29–31]. MAAH typically requires only 10–50 pmol of pure protein, and the whole procedure is rapid and easy to operate. The protein is subjected to a brief (0.5–2 min) acid hydrolysis with the assistance of microwave irradiation [30]. Mass analysis of the resultant polypeptide ladder by MALDI-MS allows each amino acid to be read from only one spectrum. It should be noted that the MAAH-MALDI technique is developed for sequencing proteins and analyzing protein modification, not for protein profiling in a complex proteome sample (i.e., to identify as many proteins as possible). While the technique can provide detailed knowledge on protein sequence and modification, it requires a purified protein to generate unambiguous sequence information. Thus, to analyze complex protein mixtures prior, protein separation by techniques such as LC or CE is required.

Previously, we demonstrated that preparative CZE can be performed with 100 μm i.d. capillaries [33] using semi-permanent double chained cationic surfactant coatings to prevent protein adsorption [34–37]. However, injection of high protein concentrations can cause significant electromigration dispersion which significantly broadens the CZE peaks [33]. This broadening can be reduced by using high buffer concentrations and/or buffer co-ions that match the protein mobility. This work investigates the collection and isolation of picomole quantities of pure protein using a commercial CE instrument for subsequent MAAH/MALDI-MS. The impact of various strategies of increasing CZE loading on MAAH/MALDI-MS are investigated.

2. Experimental

2.1. Apparatus

A Beckman-Coulter P/ACE^{TM} MDQ instrument (Fullerton, CA, USA) equipped with a UV absorbance detector was used for all protein separations and fraction collection. Proteins were detected at 214 nm with lithium phosphate buffer and at 254 nm for the Bis–tris phosphate buffer. Untreated fused-silica 100 μm i.d./363 μm o.d. capillaries (Polymicro Technologies, Phoenix, AZ, USA) with a total length of 58.0 cm (48.0 cm to the detector) were used. The capillary was thermostatted at 25 °C. Data acquisition (4 Hz) and control were performed using P/ACE station software (Version 2.3; Beckman) on a 300-MHz IBM personal computer. 0.2 mL Polymerase chain reaction (PCR) tubes (Rose Scientific Ltd., Edmonton, AB, Canada) with their caps detached were used for fraction collection.

Mass spectral measurements were performed on a Bruker Reflex III MALDI-TOF mass spectrometer equipped with a SCOUT 384 multiprobe inlet (Bremen/Leipzig, Germany). The instrument is equipped with a pulsed nitrogen laser at 337 nm. Delayed ion extraction was applied to improve the mass resolution of the linear time-of-flight mass spectrometry (TOF MS). The spectra were externally calibrated using bovine heart cytochrome *c* (b-cyt *c*, C3131, M_r = 12230.70 Da) as a mass standard. All data were processed with Igor Pro Software package (WaveMetrics, Lake Oswego, OR, USA). The mass spectra shown were normalized to the most intense signal in the displayed mass range.

2.2. Materials and reagents

Nanopure 18-MΩ ultra-pure water (Barnstead, Dubuque, IO, USA) was used to prepare all solutions. Lithium phosphate buffer was prepared from phosphoric acid (BDH, Darmstadt, Germany), and the pH was adjusted using lithium hydroxide (BDH). Bis–tris phosphate buffer was prepared from phosphoric acid, using a 100 mM solution of ultra-pure tris(hydroxylmethyl)aminomethane bis(2-hydroxyethyl)iminotris(hydroxyl-methyl)methane (Bis–tris; Sigma, St. Louis, MO, USA) to adjust the pH at 7.0.