Analysis of Peptides, Proteins, Protein Digests, and Whole Human Blood by Capillary Electrophoresis/Electrospray Ionization-Mass Spectrometry Using an In-capillary Electrode Sheathless Interface

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An in-capillary electrode sheathless interface was applied to the capillary electrophoresis/electrospray ionization-mass spectrometry (CE/ESI-MS) analysis of mixtures of small peptides, proteins, and tryptic digests of proteins. The effects of different experimental parameters on the performance of this CE/ESI-MS interface were studied. The distance of the in-capillary electrode from the CE outlet and the length of the electrode inside the capillary had no significant effects on the CE separation and ESI behavior under the experimental conditions used. However, significant enhancement of the sensitivity resulted from the use of narrower CE capillaries. Using a quadrupole mass spectrometer, an aminopropylsilane-coated capillary, and a wide scan mass-to-charge ratio range of 500–1400, detection limits of approximately 4, 1, and 0.6 fmol for cytochrome c and myoglobin were achieved for 75-, 50-, and 30-μm inner diameter capillaries, respectively. Approximately one order of magnitude lower detection limits were achieved under the multiple-ion monitoring mode. The application of the in-capillary electrode sheathless interface to real-world samples was demonstrated by CE/ESI-MS analysis of a human blood sample. (J Am Soc Mass Spectrom 1998, 9, 1081–1088) © 1998 American Society for Mass Spectrometry

Since Fenn and co-workers [1, 2] reported the observation of multiply charged species using electrospray ionization (ESI), mass spectrometry (MS) has revolutionized the analysis of biological samples. Parallel with the development of electrospray ionization, during the last decade, modern capillary electrophoresis (CE) has also rapidly matured into an effective and widely accepted analytical technique [3], having a number of practical advantages over conventional-scale analytical separation methods: high separation efficiencies (up to $10^7$ theoretical plates), rapid separation, and economy of sample size (a few nanoliters or less) [4]. The combination of a high-efficiency separation technique such as CE with the compound identification capability of mass spectrometry provides a powerful system for the analysis of complex biological mixtures [5].

Currently, two types of CE/ESI-MS interfaces are most commonly used to couple capillary electrophoresis to mass spectrometry. These are sheath-flow and sheathless CE/ESI-MS interfaces [6–12]. We recently reported a sheathless interface for CE/ESI-MS using an in-capillary electrode [9]. This interface was fabricated by inserting a small platinum (Pt) wire into the capillary through a small hole near its outlet to create an electrical connection to the buffer solution. Advantages of this new design include (1) a stable CE and ESI current, (2) durability, (3) a reduced risk of sparking between the capillary tip and the inlet of the mass spectrometer, (4) lack of dead volume, and (5) facile fabrication with common tools and chemicals. For example, the combination of the in-capillary electrode interface with a sharpened glass capillary tip allows the capillary to be placed very close to the inlet of the mass spectrometer, resulting in maximum ion transport into the mass spectrometer. As a result, a detection limit [signal-to-noise ratio ($S/N$) = 3] of approximately 4 fmol was achieved for myoglobin under a wide scan mass-to-charge ratio ($m/z$) range of 550–1400 utilizing a 75-μm inner diameter (i.d.) aminopropylsilane (APS)-treated CE capillary.

Here we report a significant enhancement in the sensitivity of this interface compared to our previous report through the utilization of smaller i.d. capillaries. In addition, the effects of different experimental parameters on the performance of this CE/ESI-MS interface are discussed. The parameters investigated include the
the addition of a second stage of mechanical pumping similar to the design recently reported by this laboratory [15]. The mass spectrometer was operated in either wide scan mode or in multiple-ion monitoring mode. In multiple-ion monitoring mode, 3 m/z values were selected using a mass window of 5 Da and an integration time of 0.30 s for each mass window.

The buffer solution (0.01 mol/L acetic acid, pH 3.4) was prepared using distilled water (Barnsted NANO-pure II, Boston, MA) and glacial acetic acid (Baxter Healthcare Corporation, McGaw Park, IL). The hydrochloric acid (HCl) and hydrofluoric acid (HF) solutions were purchased from Fisher Scientific Company (Fair Lawn, NJ). Nitrogen gas was purchased from Wilson Oxygen & Supply Company (Austin, TX). Modified trypsin (Promega, Madison, WI) was used for protein digestion [16]. All other chemicals including peptides, proteins, and APS were purchased from Sigma Chemical Company (St. Louis, MO) and used without further purification. Three peptide standard solutions were used in these studies. Two standard solutions (A and B) were prepared by: (A) dissolving 2.0 mg of each peptide (AESE, 362.3 Da; FV, 264.3 Da; morphiceptin, 521.6 Da; VHLTPVEK, 922.1 Da; bradykinin, 1060.2 Da; and cytochrome c, 12359.8 Da) in 10 mL of a methanol+water+acetic acid solution (47:47:6, v/v), and (B) 2.5 mg of each peptide (AESE, FV, morphiceptin, and bradykinin) in 10 mL of the same solution. The third standard (C) was Sigma’s high-performance liquid chromatography (HPLC) peptide standard, which is composed of approximately 0.125 mg of GY (238.2 Da) and 0.5 mg each of VYV (379.5 Da), methionine enkephalin (573.7 Da), leucine enkephalin (555.6 Da), and angiotensin II (1046.2 Da). Sigma’s HPLC peptide standard was dissolved in 1 mL of pure water. The protein standard (standard D) contained 0.2 mg each of β-lactoglobulin A, ribonuclease A, myoglobin, lysozyme, and cytochrome c in 1 mL of 0.01 mol/L acetic acid solution. All solutions were filtered through a 13-mm/0.5-μm syringe filter (Baxter Scientific, Grand Prairie, TX).

The tryptic digests utilized an enzyme-to-substrate ratio of approximately 1:30 in a 0.05 mol/L ammonium bicarbonate (pH 8.1) solution kept at 37°C for 16 h. The digest was then dried in a vacuum centrifuge and redissolved in a water+methanol solution (50:50, v/v) containing 3 mmol/L formic acid with an approximate sample concentration of 50 μmol/L.

Fresh human blood was obtained from a healthy adult man. Samples of this blood were prepared by diluting 4 μmol/L aliquots with 0.01% aqueous acetic acid (pH 3.4) to the following ratios: 1:15; 1:150; 1:1500, and 1:15000. No purification step was used. These samples were injected into the CE column using 1.0 psi pressure for a duration of 3 s. Assuming a 15 g/100 mL concentration of hemoglobin in normal blood [17], the amounts of hemoglobin injected into CE column were approximately 10 pmol, 1 pmol, 100 fmol, and 10 fmol, respectively, for the diluted blood. The blood experiments were performed on a newly acquired Mariner