On-Line Capillary Electrophoresis with Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

Steven A. Hofstadler, Jon H. Wahl, James E. Bruce, and Richard D. Smith*

Chemical Sciences Department and Molecular Sciences Research Center Pacific Northwest Laboratory Richland, Washington 99352

Received April 7, 1993

The role of capillary electrophoresis (CE) in chemical and biochemical characterization is growing rapidly due to its speed, resolution, and flexibility for the manipulation of extremely small samples.[1] CE was first combined with mass spectrometry (MS) at our laboratory,[2] and this combination has continued to attract growing attention.[3-5] Most on-line CE–MS reported to date has been based upon electrospray ionization (ESI) interfaces, although continuous flow fast atom bombardment interfaces have also been effectively used.[6] One advantage of the ESI interface is that very large molecular species can be detected at relatively low m/z due to multiple charging;[7] consequently, the necessary m/z range that must be examined is effectively reduced by a factor corresponding to the number of charges.[2] Extraordinary detection sensitivity is also possible using CE–MS; we have recently demonstrated that low-resolution mass spectra could be obtained for proteins injected at subfemtomole levels using a quadrupole mass spectrometer,[8] based upon the use of small-i.d. capillaries. Generally, however, most CE applications require detectable amounts in the femtomole range and below,[9] and with conventional scanning mass spectrometers a compromise is invariably faced between factors that include m/z scan range, MS resolution, and sensitivity. Typically, resolution, scan range, or scan speed is compromised to obtain sufficient detection limits. In this communication we report results for the first on-line combination of CE with Fourier transform ion cyclotron resonance (FTICR) mass spectrometry, an approach that is capable of providing *both* high CE and MS resolution and high sensitivity.

The advantages of FTICR include the ability to simultaneously realize ultrahigh MS resolution/mass measurement accuracy and high sensitivity, as well as the capability for higher order tandem MS methods (i.e., MS^n, where $n \geq 2$) for structural studies because of its nondestructive detection method.[10] The combination of ESI with FTICR was pioneered by McLafferty and co-workers,[11] who have demonstrated that very high resolution ($>10^6$) can be obtained for proteins.[12] We have recently developed new ESI–FTICR instrumentation incorporating features that allow rapid manipulation of pressures in the FTICR cell between those that appear optimum for ion trapping and cooling (i.e., $>10^{-5}$ Torr) and those for high-resolution detection ($<10^{-8}$ Torr).[13] The features of this instrumentation include five differentially pumped regions, two high-speed shutters to enhance differential pumping in regions close to the ESI source, and an integral cryopump that extends into the bore of the 7-T superconducting magnet that provides effective pumping speeds of $>10^5$ L/s in close proximity to the trapped ion cell.

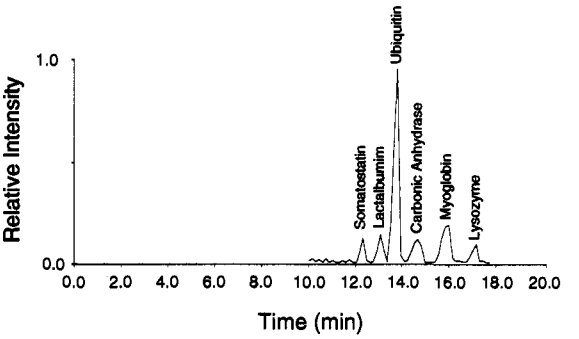

Figure 1. Total ion electropherogram for a CE–FTICR separation of a mixture of a polypeptide and five proteins. The separation was conducted in a 100-μm-i.d. capillary using a 10 mM acetic acid buffer. Electromigration was used to inject approximately 500 fmol/component into the CE capillary.

Our initial on-line CE–FTICR separations have used both 100- and 20-μm-i.d. capillaries with mixtures of standard polypeptides and proteins.[14] The FTICR experimental sequence involves injection of ions from the external ESI source using rf quadrupole ion guides. Ions traversing the trapped ion cell are collisionally trapped and cooled using a pulsed gas (N_2) inlet.[13] The ESI source utilized a heated metal capillary inlet maintained at ~150 °C. Figure 1 shows a total ion electropherogram (m/z 800–1800) obtained using a 100-μm-i.d. capillary for a CE injection corresponding to approximately 500 fmol/component. Mass spectra were obtained every 6 s.[15] Figure 2 shows mass spectra for three of the components from this separation, and inserts show detail for specific peaks demonstrating MS resolution sufficient to resolve the 1-Da spacing of the isotopic envelope is

* Author to whom correspondence should be addressed.

(1) (a) Jorgenson, J. W. *ACS Symp. Ser.* **1987**, *335*, 182. (b) Walbroehl, Y.; Jorgenson, J. W. *J. Microcolumn Sep.* **1989**, *1*, 41. (c) Kennedy, R. T.; Oates, M. D.; Cooper, B. R.; Nickerson, B.; Jorgenson, J. W. *Science* **1989**, *246*, 57.

(2) (a) Olivares, J. A.; Nguyen, N. T.; Yonker, C. R.; Smith, R. D. *Anal. Chem.* **1987**, *59*, 1230–1232. (b) Smith, R. D.; Olivares, J. A.; Nguyen, N. T.; Udseth, H. R. *Anal. Chem.* **1988**, *60*, 436–441.

(3) Lee, M. D.; Mück, W.; Henion, J. D.; Covey, T. R. *J. Chromatogr.* **1988**, *458*, 313–321.

(4) Moseley, M. A.; Shabanowitz, J.; Hunt, D.; Tomer, K. B.; Jorgenson, J. W. *J. Am. Soc. Mass Spectrom.* **1992**, *3*, 289–300.

(5) Pleasance, S.; Thibault, P.; Kelly, J. *J. Chromatogr.* **1992**, *591*, 325–339.

(6) (a) Moseley, M. A.; Deterding, L. J.; Tomer, K. B.; Jorgenson, J. W. *Rapid Commun. Mass Spectrom.* **1989**, *3*, 87–93. (b) Reinhold, N. J.; Niessen, W. M. A.; Tjaden, U. R. *Rapid Commun. Mass Spectrom.* **1989**, *3*, 348–357. (c) Moore, W. T.; Caprioli, R. M. *Techniques in Protein Chemistry II*; Academic Press: New York, 1991; pp 511–528.

(7) Smith, R. D.; Loo, J. A.; Edmonds, C. G.; Barinaga, C. J.; Udseth, H. R. *Anal. Chem.* **1990**, *62*, 882–899.

(8) Wahl, J. H.; Goodlett, D. R.; Udseth, H. R.; Smith, R. D. *Anal. Chem.* **1992**, *64*, 3194.

(9) Smith, R. D.; Loo, J. A.; Ogorzalek Loo, R. R.; Busman, M.; Udseth, H. R. *Mass Spectrom. Rev.* **1991**, *10*, 359–451.

(10) (a) Buchanan, M. V.; Hettich, R. L. *Anal. Chem.* **1993**, *65*, 245A. (b) Köster, C.; Kahr, M. S.; Castoro, J. A.; Wilkins, C. L. *Mass Spectrom. Rev.* **1992**, *11*, 495.

(11) Henry, K. D.; Quinn, J. P.; McLafferty, F. W. *J. Am. Chem. Soc.* **1991**, *113*, 5447.

(12) Beu, S. C.; Senko, M. W.; Quinn, J. P.; McLafferty, F. W. *J. Am. Soc. Mass Spectrom.* **1993**, *4*, 190.

(13) Winger, B. E.; Hofstadler, S. A.; Bruce, J. E.; Udseth, H. R.; Smith, R. D. *J. Am. Soc. Mass Spectrom.*, in press.

(14) The analytes chosen for the initial study were somatostatin (1638 Da), ubiquitin (8565 Da), α-lactalbumin (14 175 Da), lysozyme (14 306 Da), myoglobin (16 951 Da), and human carbonic anhydrase I (28 802 Da), each at approximately 50 μM. Samples were electrokinetically injected at ~−35 V cm^{-1}, and amounts injected were determined on the basis of the electromigration rates of the components. The analytical capillaries used were approximately 1 m in length and 100 and 20 μm in i.d., and the separations were performed at approximately 200 V cm^{-1} where, for the 100-μm-i.d. case, the elution rate was reduced by half prior to elution of the analytes. The inner surfaces of the capillaries were treated with aminopropylsilane to reduce interactions of analytes with the capillary inner surface.[4] The CE buffer was a 10 mM acetic acid solution (pH 3.4). The ESI interface was a sheathless design using a metalized capillary terminus, similar to that used for early CE–MS studies.[2]

(15) The mass spectrum acquisition rate was limited by the combination of ion injection, ion cooling, vacuum pump down in the cell region, and an additional data storage period required by our present data system. Thus, the MS sensitivity, resolution, and scan speed obtained for these studies should not be taken as the best achievable with the present instrumentation. On average, a single mass spectrum required <2.5 s for completion: 100 ms for ion injection (concurrent with a 10–50-ms pulsed N_2 inlet), 1.9 s for ion cooling/pump down, and 0.3–0.4 s for transient acquisition. The remaining delay between scans is due to data formatting and storage time.

0002-7863/93/1515-6983$04.00/0

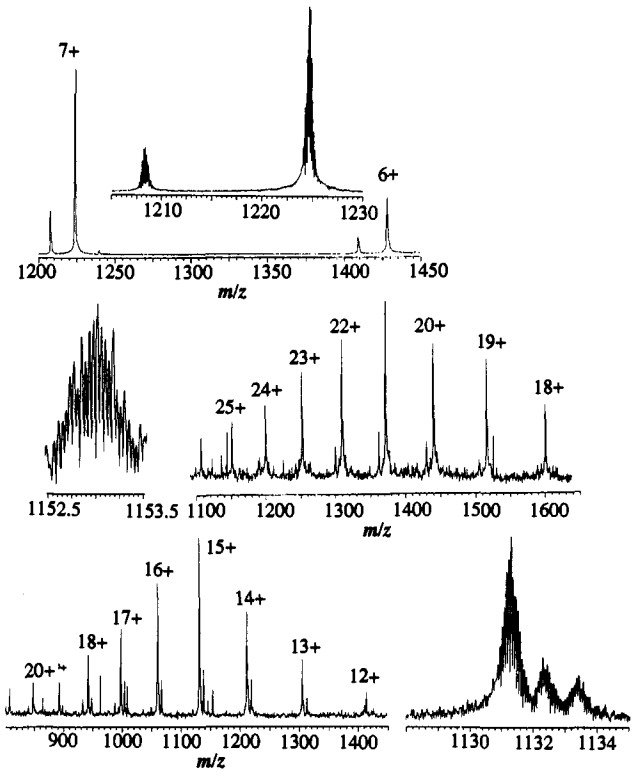

Figure 2. FTICR mass spectra for three proteins (top to bottom ubiquitin, carbonic anhydrase I, and myoglobin) from the separation shown in Figure 1. The inserts show detail demonstrating MS resolution of the 1-Da spacing of the isotopic envelope for the multiply charged proteins.

achieved, even for the largest protein studied (carbonic anhydrase I, 28 802 Da).[16] The mass spectra (Figure 2) yielded an average resolution in excess of 30 000, demonstrating the unique combination of sensitivity and high MS resolution afforded by CE–FTICR. Figure 3 shows an initial separation obtained using a 20-μm-i.d. capillary where the injected sample size corresponds to approximately 6 fmol/component. The mass spectrum in Figure 3 shows that both good resolution and good sensitivity are obtained. The present detection limits correspond to $\sim 10^{-6}$ M sample concentration, but the use of electrophoretic techniques to concentrate sample during injection[17] can lead to substantially improved detection limits. Even more encouraging is the fact that the small ion injection time (and duty cycle) used in the pulse sequence corresponds to an average of only 20 amol of sample consumed per spectrum.

The present results represent the first *on-line* combination of CE with FTICR, as well as the first on-line CE with high MS

(16) Resolution is sufficient to resolve the 1-Da isotopic spacing for molecular weights up to approximately 30 000 in the present studies. Resolving power in excess of 150 000 has been achieved utilizing slower scan speeds.

(17) Thompson, T. J.; Foret, F.; Vouros, P.; Karger, B. L. *Anal. Chem.* **1993**, *65*, 900.

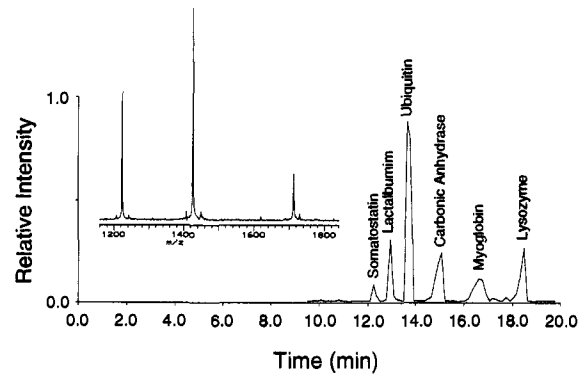

Figure 3. CE–FTICR ion electropherogram and mass spectrum obtained using a 20-μm-i.d. CE capillary into which approximately 6 fmol/component was injected by electromigration. The mass spectrum for bovine ubiquitin shows that good MS sensitivity and resolution are obtained during the separation. Resolution of the 1-Da spacing of the isotopic envelope was obtained for all peaks.

resolution detection. Wilkins and co-workers[18] have recently demonstrated off-line CE–FTICR using matrix-assisted laser desorption/ionization (maldi) where minimum CE injection sizes were ~ 5 pmol, and MS resolution for the singly charged molecules with CE was insufficient to resolve the 1-Da spacing of isotopic constituents. Several advantages of ESI relative to maldi with FTICR are evident: on-line combination is facilitated, and the multiple charging of ions allows detection at lower m/z, but relatively high molecular weight, with greater MS resolution and sensitivity. The high pumping speed afforded by our cryopumping arrangement is crucial to the relatively rapid mass spectral acquisition rates obtained in this work. The present results clearly show that CE–FTICR can be a powerful research tool, combining the flexibility and high resolution of CE with the high-performance MS characteristics intrinsic to FTICR. Efforts are in progress to further enhance the performance of this instrumental combination, as well as to use collisionally activated dissociation methods for gaining structural information for large biomolecules.[19,20]

Acknowledgment. We thank the U.S. Department of Energy and Laboratory Directed Research and Development of Pacific Northwest Laboratory for support of this research. Pacific Northwest Laboratory is operated by Battelle Memorial Institute for the U.S. Department of Energy, through Contract No. DE-AC06-76RLO 1830.

(18) Castoro, J. A.; Chiu, R. W.; Monnig, C. A.; Wilkins, C. L. *J. Am. Chem. Soc.* **1992**, *114*, 7571.

(19) Loo, J. A.; Quinn, J. P.; Ryu, S. I.; Henry, K. D.; Senko, M. W.; McLafferty, F. W. *Proc. Natl. Aca. Sci. U.S.A.* **1992**, *38*, 286.

(20) (a) Loo, J. A.; Edmonds, C. G.; Smith, R. D. *Anal. Chem.* **1991**, *63*, 2488–2499. (b) Barinaga, C. G.; Edmonds, C. G.; Udseth, H. R.; Smith, R. D. *Rapid Commun. Mass Spectrom.* **1989**, *3*, 160–164. (c) Smith, R. D.; Loo, J. A.; Barinaga, C. J.; Edmonds, C. G.; Udseth, H. R. *J. Am. Soc. Mass Spectrom.* **1990**, *1*, 53–65. (d) Loo, J. A.; Edmonds, C. G.; Smith, R. D. *Anal. Chem.* **1993**, *65*, 425–438.