

Recent Developments in Microchip ESI MS for Protein Analysis

R. S. Ramsey, I. M. Lazar, N. Gottschlich, and J. M. Ramsey
Oak Ridge National Laboratory
Chemical and Analytical Sciences
Oak Ridge, TN 37831-6365

We have developed a microchip electrospray source that provides sub-fmole sensitivity for peptides and proteins using time-of-flight detection. The device consists of a disposable nanospray tip coupled to a planar glass chip with microfabricated channels. We have also identified conditions suitable for rapid on-chip tryptic digestion and subsequent electrospray MS analysis for protein identification using peptide mass fingerprinting. The fragments are generated in basic buffer at the optimum pH for proteolytic cleavage using relatively high substrate to enzyme ratios to accelerate the reaction. Cleavage products are then determined directly in the digestion mixture using “wrong-way-round” electrospray (i.e., monitoring $M+H^+$ ions from the basic solution). Sample and enzyme, mixed off-chip, are immediately loaded into a sample reservoir, and then electrosprayed by applying voltage to the inlet port. Intense tryptic peptide ions are observed within a few minutes following the onset of electrospray. Fragments have also been generated by introducing discreet volumes of sample and enzyme contained in separate ports on a microchip with diffusional mixing in a channel. Mass spectra have been produced from low fmole quantities/spectrum of protein (consumed). The method has been used to analyze human blood extracts for hemoglobin where sequence coverage of 70 to 95% was obtained. Sick cell hemoglobins have been rapidly identified by monitoring mutated vs. normal fragments.

Research at the Oak Ridge National Laboratory sponsored by the National Cancer Institute, U.S. Department of Energy, under contract No. DE-AC05-00OR22725 with Oak Ridge National Laboratory, managed and operated by UT-Battelle, LLC.

“The submitted manuscript has been authored by a contractor of the U.S. Government under contract No. DE-AC05-00OR22725. Accordingly, the U.S. Government retains a paid-up, nonexclusive, irrevocable, worldwide license to publish or reproduce the published form of this contribution, prepare derivative works, distribute copies to the public, and perform publicly and display publicly, or allow others to do so, for U.S. Government purposes.”