Using Poroshell 300SB-C18 for High-Sensitivity, High-Throughput Protein Analysis on the Agilent LC/MSD

Application

Proteomics

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In the modern protein chemistry laboratory, time and sample are usually in short supply and cannot be wasted. The Poroshell 300SB-C18 column allows the protein analyst to both shorten analysis time and increase method sensitivity. The mass spectra shown in Figures 1 and 2 demonstrate that high-speed analysis with excellent sensitivity is possible. Also sensitive molecular weight (MW) determinations by deconvolution analysis are possible, even under conditions of incomplete chromatographic separation and the presence of stabilizers, as shown in Figure 2, or in tissue culture media, as shown in Figure 3. These examples demonstrate the utility of using Poroshell 300SB-C18 with the high sensitivity of the Agilent liquid chromatograph/mass selective detector (LC/MSD) for fast protein molecular weight determinations.

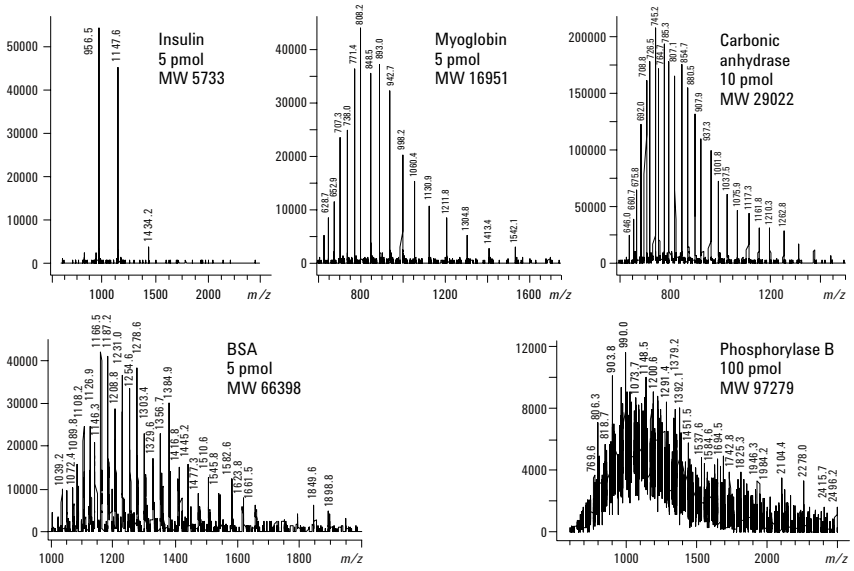

Figure 1. Mass spectra of several proteins using formic acid.

In electrospray ionization (ESI), the more charge states for a protein, the poorer the sensitivity because the signal is divided over more ions. Due to the complexity of overlapping charge envelopes, it may not be possible to determine the molecular weight for heterogenic proteins without chromatographically separating the isoforms (see Phosphorylase B in Figure 1).

Highlights

- Poroshell was designed to support the needs of the protein chemist - high speed, high sensitivity, and superior resolution in one small package.
- Formic acid modifier gives acceptable chromatographic performance on the Poroshell column and greatly enhances the MS sensitivity compared to trifluoroacetic acid (TFA) modifier (data not shown)
- Because of the thin superficially porous layer, Poroshell 300SB-C18 allows for rapid equilibration, especially for large molecules exhibiting slower diffusion into pores.
- Poroshell columns allow for rapid MS analysis of intact proteins even in the presence of stabilizers and tissue culture media.
- MW determination may be accomplished with as little as 1–5 pmol of protein on a standard Poroshell column.

Experimental Conditions

Column: ZORBAX Poroshell 300SB-C18, 75 mm long x 2.1 mm id, 5-µm particles, 60 °C; **Mobile phase:** A: water with 0.1% formic acid; B: acetonitrile with 0.1% formic acid; **Gradient:** 20 to 100% B in 5.5 minutes; **Flow rate**: 500 µL/min; **Injection volume:** 1 µL; **Diode array detector (DAD):** signal 220/4 nm; **Electrospray ionization (ESI) mode:** positive ion; **V_{cap}:** 6000 V; **Drying gas:** 12 L/min; 350 °C; **Nebulizer:** 45 psi; **Fragmentor voltage:** 140 V; **Scan:** 600-2500 amu; **Step size:** 0.15 amu; **Peak width:** 0.06 min

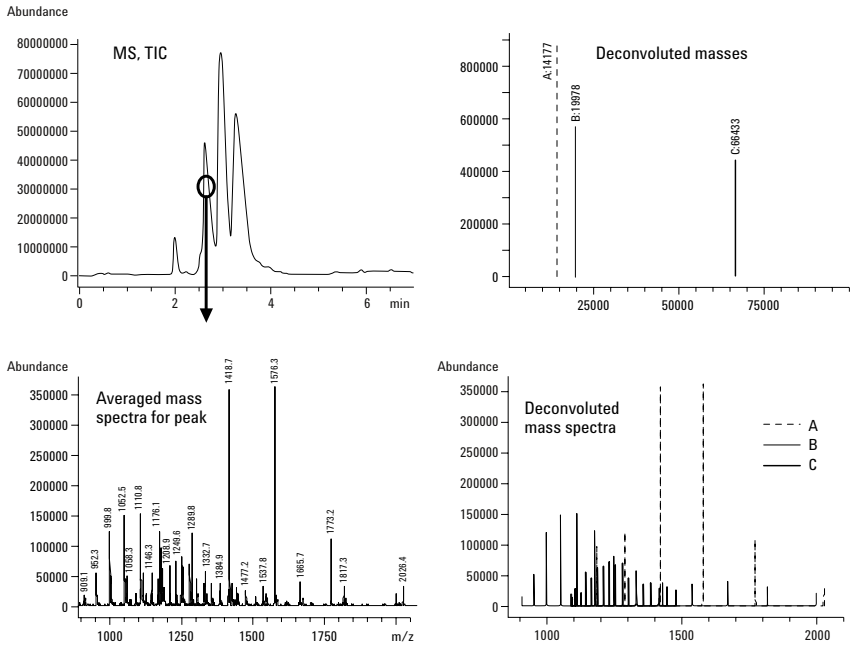

Figure 2 Analysis of a standard protein mixture.

A low-MW standard (Pharmacia), containing six proteins plus the stabilizer, sucrose, was separated in 4 minutes. Even though all proteins were not completely separated (for example, peak at 2.6 min), automatic deconvolution analysis resolved the components and obtained their respective molecular weights and mass spectra, as shown on the right side of Figure 2. The deconvoluted mass spectra show three proteins for this peak: (A): α-lactalbumin (14 kD), (B): soybean trypsin inhibitor (20 kD), and (C): bovine serum albumin (BSA, 66 kD).

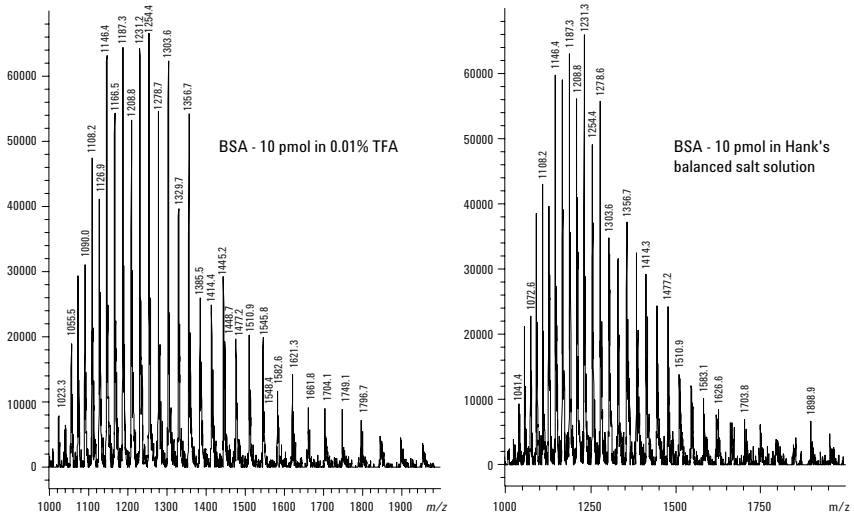

Figure 3. Analysis of bovine serum albumin in the presence of tissue culture media.

BSA was chromatographed using both TFA and Hank's balanced salt solution to separate the salts from the protein. Identical charge envelopes and MW were produced, without loss of sensitivity, as shown in Figure 3.

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