Rapid Proteomic Sample Preparation for Sensitive, Reproducible, High-throughput MALDI-MS Analyses

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Introduction: Biological samples can be studied for biomarker discovery through the analysis of their protein and peptide constituents by rapid and high-throughput MALDI-TOF MS. Large-scale proteomic analyses of complex sample mixtures, however, are very time consuming due to the multi-dimensional fractionation and other sample preparation procedures that necessarily precede MS analysis. The resulting sample fractions are often dilute and require multiple steps of sample handling (e.g., digestion, de-salting, transfer, sample concentration), which are potential sources of contamination and loss. We have developed simple, inexpensive (non-robotic) 96-well array devices to conduct high-throughput sample preparation for MALDI-MS analysis. We employ this technology for the efficient coupling of 2D protein and peptide LC directly to MALDI-TOF MS.

Methods: Representative peptide and protein standards were used to develop sample preparation conditions for biological samples. Samples were concentrated under vacuum and co-crystallized with matrix. The amounts of peptide samples subjected to analysis ranged from 4 pmol to 4 fmol. Several re-focusing steps with different organic solvent mixtures were employed to aid sample concentration. Additionally, various procedures were tested to improve on-target matrix crystallization. MALDI-TOF MS data were obtained with a Bruker Reflex-IV operated in linear and reflectron modes. Results were compared to standards prepared by conventional methods. Comparisons were based on overall spectrum signal yield, signal-to-noise ratio, reproducibility, and % sample recovery. In addition to pre-made peptide mixtures, proteins were digested in the 96-well array and concentrated directly onto the target. Lastly, optimized protocols were developed for facile coupling with one- and two-dimensional HPLC fractionation of peptides and proteins obtained from a variety of sample classes, including human plasma.

Results: On-target concentration and sample/matrix co-crystallization under optimized conditions yielded reproducible and sensitive MS analyses with minimal sample loss from both peptides, proteins, and protein digests. High-quality MS data with high S/N were obtained from as little as 4 fmol of peptide standard from up to 100-µl volumes of starting solution, corresponding to acquisition of reliable MS signals even from solutions that had peptide concentration(s) at the attomolar level. Highly reproducible MS data with equivalent sequence coverage were obtained across the entire peptide dilution series ranging from 4 pmol through 4 fmol of starting material, with the absolute signal being linearly proportional to the initial sample molar amount. This sensitivity was achieved only after optimization of several re-focusing steps with organic solvent mixtures. Similarly, protein standards concentrated onto the target yielded good spectra from as little as 1 pmol of protein standard. High quality spectra were also obtained from on-target trypsin digests of bovine catalase. Direct coupling to peptide and protein HPLC allowed for the application of this approach to sample preparation of complex mixtures.

Conclusions: Overall, we have demonstrated rapid and facile sample preparation and direct coupling to HPLC MALDI-TOF MS that results in successful characterization of both peptides and proteins from complex samples. Such coupling and partial automation offers the benefit of minimal sample handling and loss while maintaining signal from low-level peptides and proteins. This approach is applicable to both peptide mass fingerprinting (PMF), as well as intact protein analysis. The encouraging results provide motivation for developing simple instrumentation that enables online HPLC-MALDI-TOF MS.

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Figures:

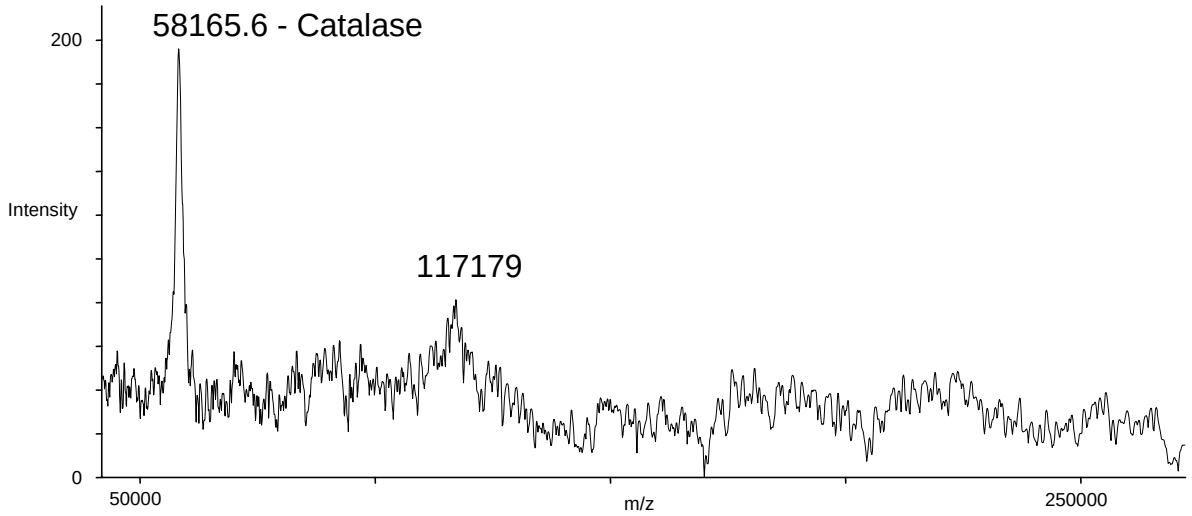

Figure 1. MALDI-TOF MS spectrum of bovine catalase. The bovine catalase was added to one of the wells on the 96-well plate and concentrated onto the target before digestion (see below).

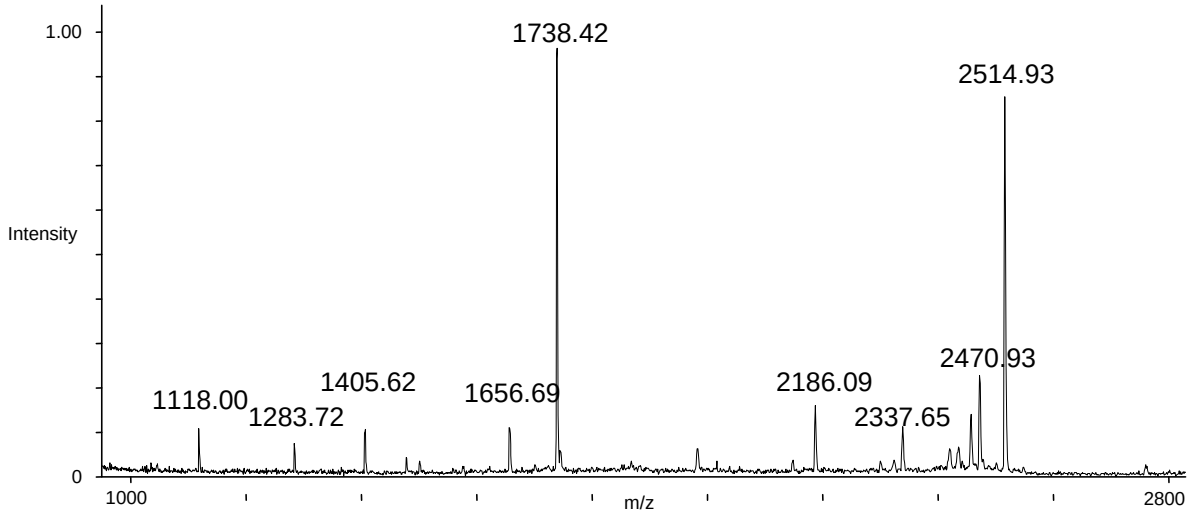

Figure 2. Spectra of a bovine catalase in-well tryptic digest obtained by MALDI-TOF MS. Digests were conducted with 100 ng of trypsin in 5 mM ammonium bicarbonate.