HIGH THROUGHPUT PROTEIN IDENTIFICATIONS UTILIZING MICROWAVE PROTEIN DIGESTS AND OFF-LINE NANO-SPRAY CHIP TECHNOLOGY

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Abstract:

We have developed a high throughput protein digestion and analysis procedure using microwave power (CEM Inc.) and an automated chip based nanospray (Advion Bioscience). Applying this method to several standard proteins revealed that the combined technologies provide highly confident identifications in a fraction of the time for both protein solutions and gel plugs. The application of the CEM Discovery system and the Advion Nanomate 100 allows for the automated processing of multiple samples resulting in high-throughput data analysis and improved data quality.

Introduction:

Standard methodologies for enzymatic digestions have changed little in the past 40 years. The same process for sample incubation with trypsin, 6-16 hours, followed by a lengthy LC/MS/MS analysis, ~90 minutes/sample followed by blank, remains an industry and academic standard. Now, with the exploitation of microwave power to decrease incubation times and reaction efficiency combined with the utilization of a chip based robotic nano-spray to decrease acquisition time, a complete digest and analysis can be completed in ~ 30 minutes.

In this presentation, a novel method for protein digestions and analysis was developed to provide high throughput analysis while increasing data quality. The traditional 16hour digestion was compared to a 10minute microwave trypsin digest for protein solutions and gel plugs. Following digestion, the samples were analyzed directly with a robotic chip based nano-spray (Advion Nanomate 100) coupled to an ion trap mass spectrometer (Finnigan LCQ Deca XP plus) and compared with the traditional 90minute LC/MS/MS analysis. These samples were also analyzed by MALDI-TOF to confirm the efficiency of the digestions.

Methods:

Standard protein digest were performed overnight at 30ºC with 0.15 ug of Promega Trypsin. CEM microwave digests were carried out for 10 minutes, with a maximum temperature setting of 60ºC, a maximum power of 55 KW, 10% cooling, and 1.5 ug of Promega trypsin. Following digestion, all samples were cleaned up utilizing Millipore C18 zip tips, with gel plug samples being sonicated for 15 minutes prior to the sample clean up step.

MALDI-TOF data was acquired utilizing the Kratos Axima CFR, in reflectron mode for both PMF and PSD data. All Nanomate acquisitions were acquired with the Thermo Finnigan LCQ Deca XP plus. The method for this analysis utilized 3 data dependant MS/MS scans across 5 segments. Each segment represented a specific m/z range, 300-650, 650-1000, 1000-1350, 1350-1700 and 1700-2000.

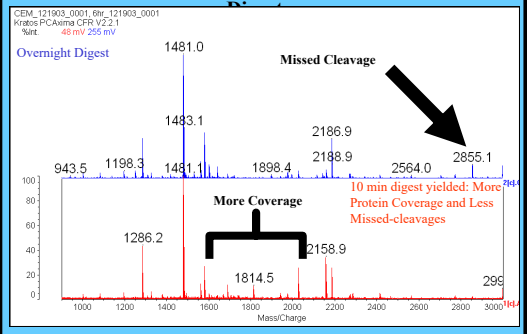
Fig. 1

CEM Discovery System

The CEM Discovery microwave unit allows for high throughput enzymatic digestions of proteins from solution samples or gel plugs in 10 minutes, while maintaining high data quality.

Fig. 3

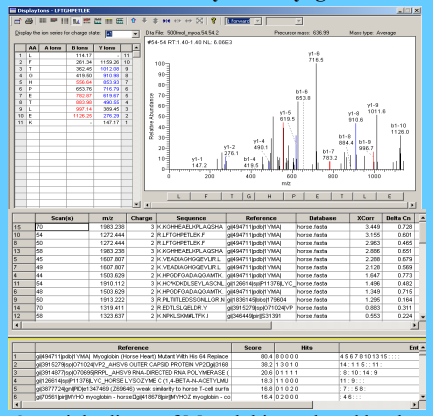
MALDI-TOF Analysis of Transferrin Solution



MALDI spectra of Transferrin. Traditional digest for 16 hrs (blue)and CEM 10 min digest (red). The 10 minute digest resulted in approximately 10% more sequence coverage and had 10% less missed cleavages.

Fig. 4

Advion Nanomate Analysis of Myoglobin: 16Hr



Overnight digest of Myoglobin analyzed by the Advion Nanomate 100. This experiment resulted in 38% sequence coverage.

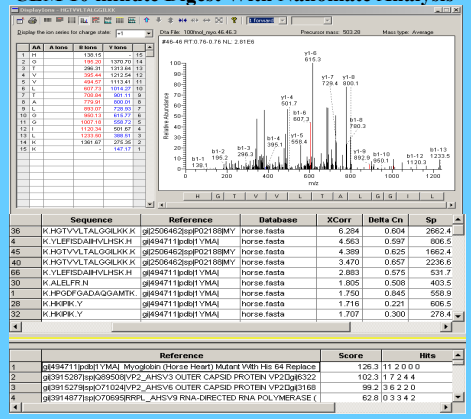
Fig. 2

Advion Nanomate 100

The Advion Nanomate 100 allows for a high throughput chip based robotic nanospray enabling automated analysis of protein digests from samples while completely eliminating carryover, reducing analysis time, and maximizing efficient utilization of the mass spectrometer.

Fig. 5

CEM 10 minute Digest With Nanomate Analysis



	Sequence	Reference	Database	XCorr	Delta Cn	Sp
36	K.HGTVVLTALGGILKK.K	gi|2506462|sp|P02188|MY	horse.fasta	6.284	0.604	2662.4
4	K.YLEFISDAIIHVLHSK.H	gi|494711|pdb|1YMA|	horse.fasta	4.563	0.597	806.5
45	K.HGTVVLTALGGILKK.K	gi|2506462|sp|P02188|MY	horse.fasta	4.389	0.625	1662.4
40	K.HGTVVLTALGGILKK.K	gi|2506462|sp|P02188|MY	horse.fasta	3.470	0.657	2236.6
66	K.YLEFISDAIIHVLHSK.H	gi|494711|pdb|1YMA|	horse.fasta	2.883	0.575	531.7
30	K.ALELFR.N	gi|494711|pdb|1YMA|	horse.fasta	1.805	0.508	403.5
1	K.HPGDFGADAQGAMTK.	gi|494711|pdb|1YMA|	horse.fasta	1.750	0.845	568.9
28	K.HKIPIK.Y	gi|494711|pdb|1YMA|	horse.fasta	1.716	0.221	606.5
32	K.HKIPIK.Y	gi|494711|pdb|1YMA|	horse.fasta	1.707	0.300	278.4

	Reference	Score	Hits
1	gi|494711|pdb|1YMA| Myoglobin (Horse Heart) Mutant With His 64 Replace	126.3	11 2 0 0 0
2	gi|3915287|sp|O71024|VP2_AHSV3 OUTER CAPSID PROTEIN VP2Dgi|3322	102.3	1 7 2 4 4
3	gi|3915279|sp|O71024|VP2_AHSV6 OUTER CAPSID PROTEIN VP2Dgi|3168	99.2	3 6 2 2 0
4	gi|3914877|sp|O70695|RRPL_AHSV9 RNA-DIRECTED RNA POLYMERASE (	62.8	0 3 3 4 2

CEM 10 minute digest of Myoglobin analyzed by the Advion Nanomate 100. This experiment resulted in 43% sequence coverage.

Conclusions:

Combination of the CEM microwave digestion and the Nanomate 100 chip based nano-spray MS/MS resulted in less missed cleavages, higher sequence coverage, and better data quality than the standard digestion protocol for solution and gel plug digestions. Improved data quality was confirmed when comparing the database search score, Xcorr values, and sequence coverage between the standard digest and 10 minute microwave approach. This combined technology provided zero carry over and only required a 5minute acquisition time per sample to achieve confident protein identifications. Advantages to this approach include high throughput sample processing and acquisition, increased data quality, and more detailed sequencing information.

Future Work:

Future method development work involving the CEM Discovery unit and Advion Nanomate 100 is currently underway. This work involves utilizing the microwave to digest whole plasma and serum for MudPIT analysis, increase ICAT labeling efficiencies, and determining the lower limits of sample required for microwave digestions out of gel plugs.