SCIENTIFIC ANALYSIS INSTRUMENTS

Application Note

LaserToF LT3 Plus

Peptide Mass Fingerprinting

Introduction

One of the most powerful applications of Matrix Assisted Laser Desorption Ionisation Time of Flight Mass Spectrometry (MALDI-TOF MS) is its ability to identify proteins. This has been made possible by the compilation of large databases which document the biochemical properties of such proteins. The approach is to correlate the data from the mass spectrometer with that held in these compilations.

The unknown protein is cleaved into peptides in a predictable manner (digested) using a protease-enzyme. Analysis of this peptide series by MALDI-TOF MS gives a characteristic mass spectrum known as a Peptide Mass Fingerprint. A theoretical digest of the primary structure of the catalogued proteins is performed in software and the resulting set of peptide masses (peptide map) is compared with the set of masses in the Peptide Mass Fingerprint; the correlation can lead to an identification of the unknown protein.

When the protease trypsin is used, the protein is cleaved specifically at arginine and lysine, giving peptides with these basic amino acids as the C-terminal residues. This is desirable for mass spectrometry because basic residues are nucleophilic, and readily protonate to facilitate ionisation and analysis.

Materials and Methods

Two proteins, alcohol dehydrogenase (ADH) from yeast (Sacchromyces cereviseae) and creatine phosphokinase from rabbit muscle were digested with the enzyme trypsin. The precise mono-isotopic mass of the peptides in each series was determined at high resolution using the LaserToF LT3 Plus.

Results and Discussion

The mass spectra obtained with the LaserToF LT3 Plus from the digested model proteins are shown in Figures 1 and 2. The Peptide Mass Fingerprints derived from these mass spectra were compared with sets of theoretical series generated from the database using the proteomics software included in the LaserToF data system. The results of the database searches are summarised in Tables 1 and 2. In both cases the correct protein has

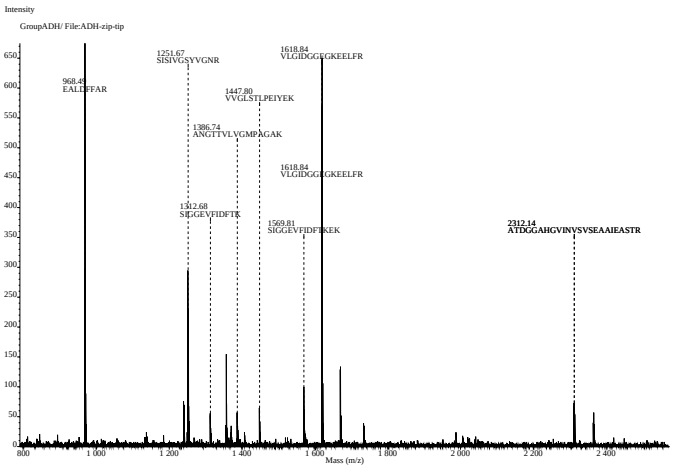

Figure 1: The Peptide Mass Fingerprint of the ADH Peptide Digest

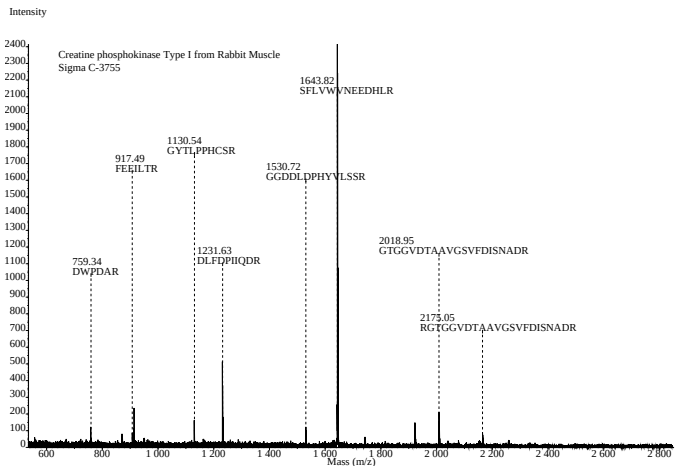

Figure 2: The Peptide Mass Fingerprint of the creatine phosphokinase Peptide Digest

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been found and the score reflects the correlation between the sets of measured and theoretical masses

The search can, if required, be constrained by taxanomic group, the mass of the protein and/or the number of residues as desired.

Table 1: Protein Identification Results for ADH

Rank	Protein	Score
1	ADH1_YEAST	200
2	COAT_BPMV4	38
3	COQ3_RAT	35
4	MTE5_ECOLI	27
5	DNJL_MYCPN	26
6	HXDA_HUMAN	26
7	HXDA_MOUSE	26
8	YQ56_CAEEL	26
9	DEK_HUMAN	24
10	ALR_AQUPY	23

Table 2: Protein Identification Results for creatine phosphokinase

Rank	Protein	Score
1	KCRM_RABIT	245
2	KCRM_CANFA	187
3	RF1_STRGC	182
4	PSD2_BOVIN	158
5	Y158_RICPR	127
6	KCRM_MOUSE	94
7	KCRM_RAT	94
8	RL21_XENLA	83
9	C2_OXYNO	69
10	KCRM_BOVIN	68

The data shown in Table 3 summarise the results of the mass correlation and illustrate how the identification of the protein is facilitated by the high mass accuracy of the LaserToF LT3 Plus system.

Table 3: Protein Identification Summary

Protein	Number of Peptides Matched	Coverage %	Mass Measurement Error (ppm)	
			Mean	RMS
ADH1_YEAST	8	29	-2.9	8.8
KCRM_RABIT	8	22	-5.7	11.2

The data presented herein demonstrate the high mass measurement accuracy and resolution which can be achieved for samples of peptide digests with the harmonic field reflectron of the LaserToF LT3 Plus. This and the local database facilitates rapid peptide mass fingerprinting.

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