ANALYSING NATURE WITHOUT COSTING THE EARTH

KRATOS ANALYTICAL
A SHIMADZU GROUP COMPANY

AUTOMATED PSD ANALYSIS OF COMPLEX PEPTIDE MIXTURES

KOMPACT

APPLICATION NOTE

AUTOMATED PSD ANALYSIS OF COMPLEX PEPTIDE MIXTURES

INTRODUCTION

MALDI MS is an established method for protein identification based on the approach of peptide fingerprinting. At the simplest level, the products of proteolytic digestion of a target protein are analysed in linear mode without prior purification or sample clean up. As only singly-charged molecules are observed, very complex mixture can be analysed. The resulting mass signals are dependant on the sequence of the protein and therefore represent a unique pattern characteristic of the original target protein. The peptide masses can then be applied to any one of a number of Web-based search engines that use protein sequence databases in order to identify the protein [(1)].

With the increasing number of protein sequences being entered into the databases, as well as putative translations from DNA fragments, the ability for ambiguous identification is also increasing. Clarification can be obtained by including post source decay (PSD) fragment data from one or more parent ions detected in the peptide spectrum. These additional masses allow unequivocal protein identification [(2)].

The Kratos reflectron MALDI systems, KOMPACT DISCOVERY and KOMPACT SEQ, are ideally suited to perform both aspects of this application using the unique patented Curved Field Reflectron [(3)] (see Figure 1) to generate both parent ion spectra and seamless PSD spectra. Further, the advances in ion gate technology (resolution ±12.5 Da at 1000 Da) enables realistic selection of parent ions, from complex mixtures, so that the whole process of MALDI fragmentation can be automated through innovative software. A tryptic digest of apomyoglobin is used here to illustrate these tools.

METHODS AND MATERIALS

Apomyoglobin (50 µg) was resuspended in 200 µl 100 mM ammonium bicarbonate, 8 M urea to give a final concentration of 10 mg/ml. The protein solution was diluted four-fold and 20 µl of modified trypsin (1 mg/ ml in water) added. The final protein:protease ratio was 25:1, in 2 M urea. After vortexing, the sample was incubated at 27°C overnight. A second addition of trypsin was made and the reaction continued for 6 hours. The reaction was stopped by freezing.

Prior to analysis the sample was diluted five-fold with water to give a final concentration of 0.4 M urea. If digestion was 100% successful, a 1 µl aliquot of sample would contain 12 pmol of each peptide product. Sample (0.5 µl) was placed on the KOMPACT target and 0.5 µl α-cyano-4-hydroxy cinnamic acid matrix (10 mg/ml in 70% acetonitrile/30% water). The mixture was allowed to dry before MALDI analysis on the KOMPACT DISCOVERY.

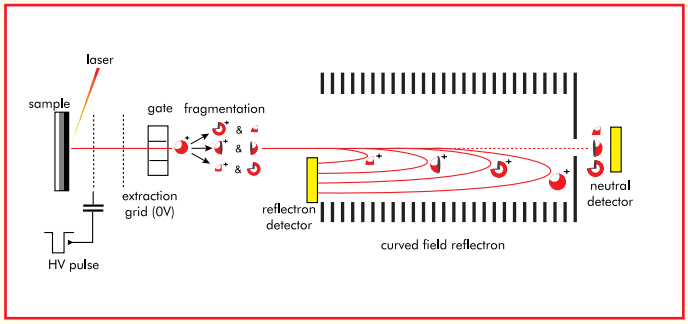

Figure 1: Schematic of the curved field reflectron

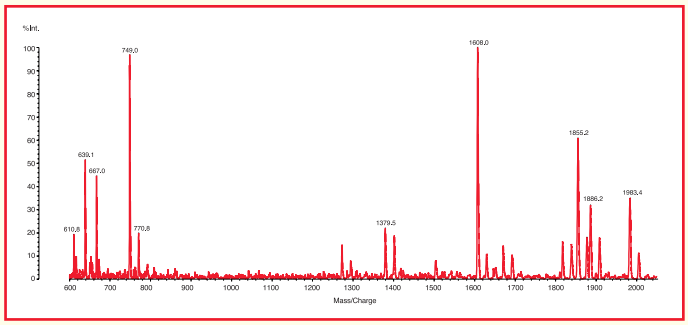

Figure 2: Parent ion spectrum of apomyoglobin digested by trypsin - Manual analysis

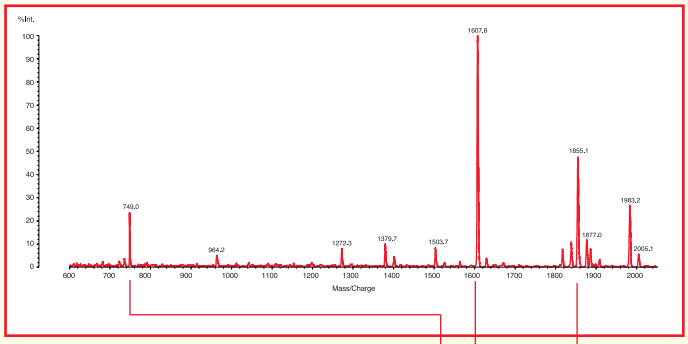

Figure 3: Parent ion spectrum of apomyoglobin digested by trypsin - Automatic analysis

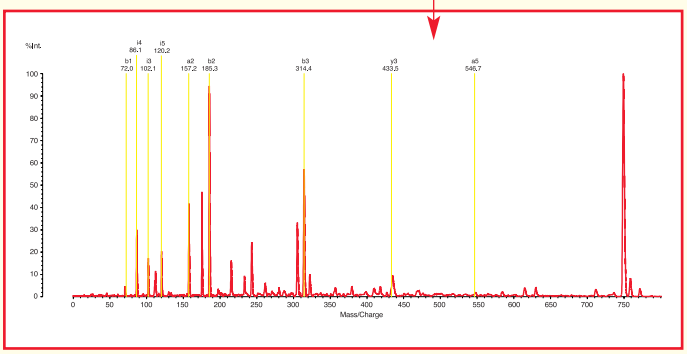

Figure 5: PSD spectrum of 749.0 Da parent ion using automatic acquisition software

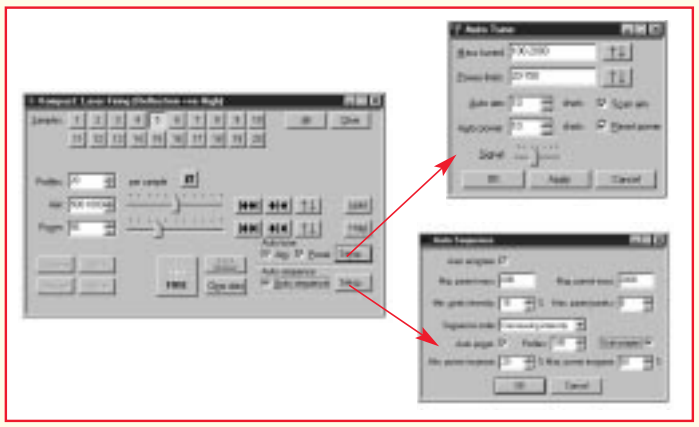

Figure 4: Window for acquisition of MALDI data, Auto Tune and Auto Sequence

Tryptic Fragment	Theoretical Masses MH^+	Sequence	Tryptic Fragment	Theoretical Masses MH^+	Sequence
1	1817.02	GLSDGEWQQVLNVWGK	12	147.20	K
2	1607.81	VEADIAGHGQEVLIR	13	1855.07	GHHEAELKPLAQSHATK
3	1272.45	LFTGHPETLIR	14	284.34	HK
4	409.46	FDK	15	470.63	IPIK
5	294.38	FK	16	1886.21	YLEFISDAIIHVLHSK
6	397.50	HLK	17	1503.64	HPGDFGADAQGAMTK
7	708.81	TEAEMK	18	748.90	ALELFR
8	662.72	ASEDLK	19	631.71	NDIAAK
9	147.20	K	20	310.37	YK
10	1379.69	HGTVVLTALGGILK	21	650.71	ELGFQG
11	147.20	K			

Table 1. Theoretical masses and sequences for the products of apomyoglobin digestion by trypsin.

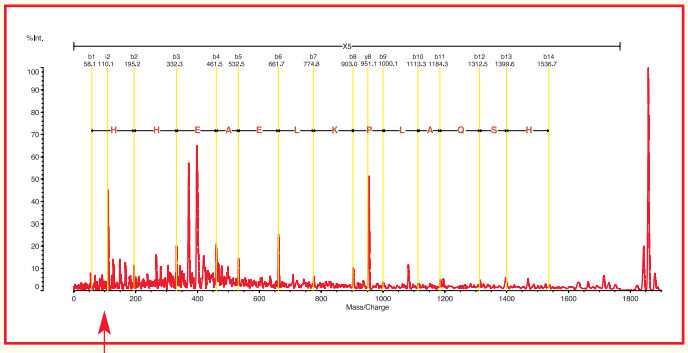

Figure 7: PSD spectrum of 1855.1 Da parent ion using automatic acquisition software

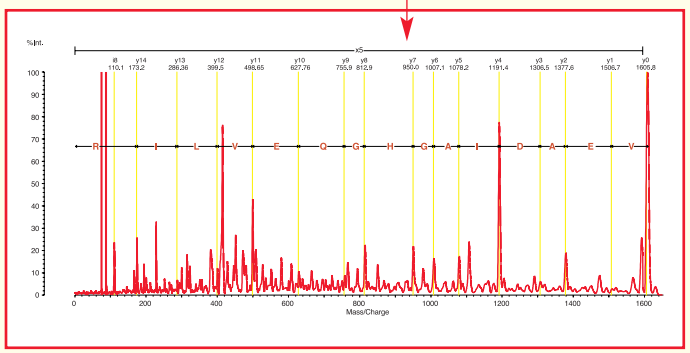

Figure 6: PSD spectrum of 1607.8 Da parent ion using automatic acquisition software

RESULTS

Spectra were readily obtained from the protease digest sample, even in the presence of 0.4 M urea (see Figure 2). The peptide signals generated represented 73 % of the total apomyoglobin sequence (Table 1). The only partial digest products observed were due to the presence of consecutive basic amino acids - ~KK~.

Spectra produced automatically (Figure 2) by the software gave equivalent results in terms of resolution and signal-to-noise as data generated manually (Figure 3). Automation was performed within easy user-defined parameters of the Auto Tune software - laser aim range, laser power range and mass range (see Figure 4).

The same principles apply to automation of PSD data acquisition in the Auto Sequence software (Figure 4). The user can define parent mass range, number of parent peaks to be analysed for PSD and the order in which parents are analysed (increasing intensity, decreasing intensity, increasing mass or decreasing mass). The ion gate is then set automatically to allow only the parent ion of interest to be analysed. The software optimises the laser power to generate the widest range of fragment ions - immonium as well as larger ones, prior to acquiring a full seamless spectrum (Figures 5 to 7).

In the example shown, 3 parents (within a predefined mass range of 600 to 1900 Da) were selected for PSD, to be analysed in order of decreasing intensity. After generating the parent ion spectrum (Figure 3), the software subsequently selected signals at 1607.8, 1855.1 and 749.0 Da for PSD analysis and generated spectra on each sequentially. The value of the new high resolution ion gate was evident in the isolation of the 1855.1 Da peak and generation of significant fragmentation, without contamination from two neighbouring parent ions at 1817.3 and 1886.1 Da (Figure 7).

The full power of the new KOMPACT Version 1.1 software can be realised in the use of post-analysis interpretation tools. Interpretation of MS/MS spectra of peptides is one of the most time consuming and difficult applications for the mass spectrometrist. New features of the KOMPACT software allow the user to make a rapid assessment of the relationship of a complex series of signals in the PSD spectra to greatly accelerate this interpretation and obtain peptide sequence information. A range of tools also allow the user various means to annotate the spectrum for preparation of reports. These were used to accelerate the processing of data from apomyoglobin peptides, and readily enabled identification of series of b or y ions in the spectra (Figure 8).

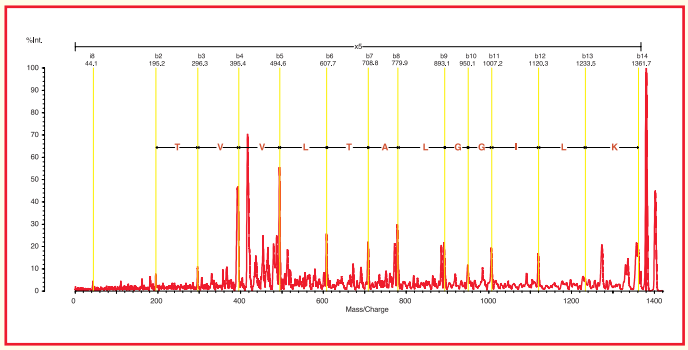

Figure 8: PSD spectrum of 1379.7 Da parent ion using automatic acquisition software

DISCUSSION

With increasing confidence the user can automate the complete process of protein and peptide structural analysis for up to 30 samples, from generation of complex tryptic peptide patterns for target proteins to preliminary sequencing of selected peptides. As an extension of this ability, it is now possible to automatically transfer data (either peptide fingerprints or fragment patterns) directly to Web-based search engines to automate protein identification [(1)].

The KOMPACT DISCOVERY and KOMPACT SEQ are ideal tools for providing significant structural information on important recombinant proteins. The software is particularly well designed for the scientist approaching MS/MS for the first time and represents a significant step forward in total automation of peptide sequencing by mass spectrometry.

BENEFITS

- Compact MALDI design without compromising performance
- Automatic sample target introduction mechanism
- Automatic generation of parent ion spectra
- Automatic setting of high resolution ion gate to select parent for PSD
- Automatic generation of seamless PSD spectra with the curved field reflectron
- Automatic annotation of the PSD spectrum to enhance sequence interpretation
- Sophisticated package of software tools for biochemical analysis

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