SHIMADZU
Excellence in Science

Application News

No. B105

MALDI-TOF Mass Spectrometry/Protein Sequencer

Protein Analysis Platform

Combining the powerful capabilities of MALDI-TOF MS (MALDI-8020) and Edman Sequencing (PPSQ™-50A Gradient System) for accurate N-terminal sequence of peptides

Introduction

Mass spectrometry has become an indispensable tool for researchers looking to sequence peptides. Although effective in many cases, sequencing by In Source Decay (ISD) faces a few challenges its ability to provide reliable sequence information including isobaric amino acids, database dependency and low molecular weight interferences.

Traditional Edman sequencing avoids mass dependency and the use of databases by analyzing each amino acid from the N-terminus one at a time in sequence. Unfortunately, Edman has its own limitations in providing high sequence coverage.

This technical note investigates the benefits of combining the intact mass and sequencing information from the MALDI-8020 (ISD) with the N-terminal sequence obtained from the PPSQ-50A gradient system (Edman). Using the combined information allows investigators the ability to obtain a more complete picture of their proteins and peptides of interest.

Combining the complementary data from PPSQ-50A gradient system and MALDI-8020 provides a more complete and accurate N-terminal sequence.

PPSQ™-50A Gradient System

MALDI-8020

Example: Analysis of Brain Natriuretic Peptide (BNP) by MALDI-8020 and PPSQ-50A Gradient System

BNP was analyzed in order to illustrate the complementary data from ISD and Edman sequencing for N-terminal sequencing. This 45 residue cyclic peptide serves as a hormone with diuretic and angiectatic effects (Fig. 1). The disulfide linkage that creates the cyclic portion of the peptide requires alkylation for analysis using the PPSQ-50A gradient system, but can be analyzed directly using MALDI-TOF MS.

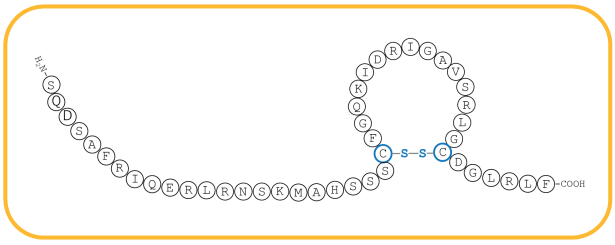

Fig. 1 Structure of Cyclic Peptide BNP

PPSQ-50A Gradient System: N-Terminal Sequencing by Edman Degradation

Edman sequencing provides conclusive N-terminal sequence information by removing the mass dependency on the identification. In a typical experiment, amino acids are removed one by one at the N-terminus of a protein through a series of derivatization and cleavage steps. High performance liquid chromatography (HPLC) is then utilized to separate the derivatized amino acids which can then be identified by the retention time on the column. Fig. 2 shows the derivatized amino acid standard that is used to determine the retention time for each amino acid. These retention times are then compared against the times recorded for each cleaved amino acid to determine its identity.

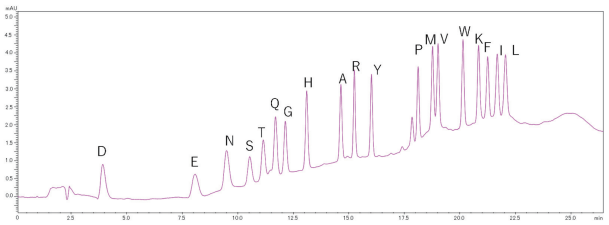

Fig. 2 Chromatogram for Derivatized Amino Acid Standard

Fig. 3 illustrates the first seven cycles of analysis for the PPSQ. After the first cycle, subsequent chromatograms can be subtracted to facilitate analysis. From the data, the N-terminus is determined to be SQDSAFR.

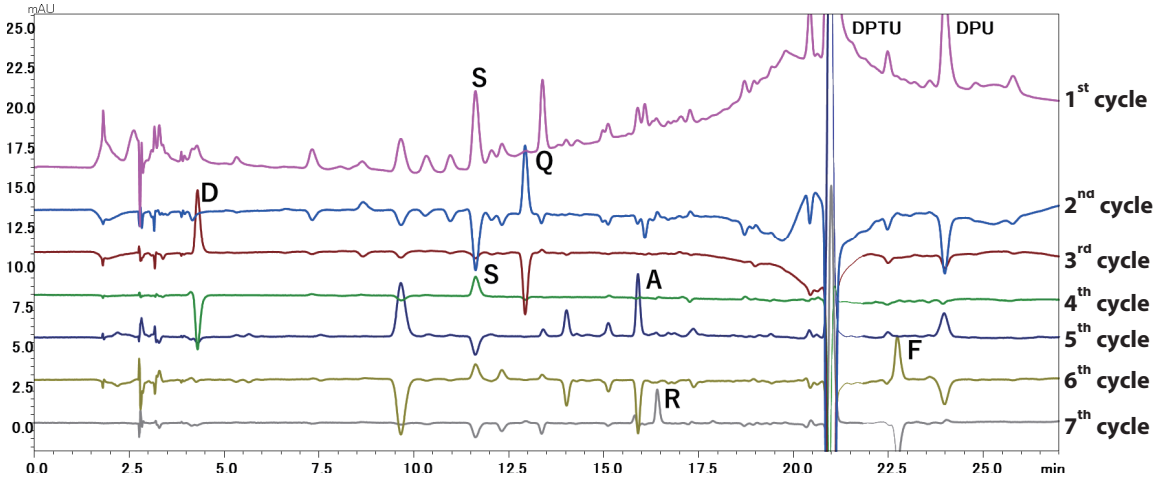

Fig. 3 Subtracted Chromatograms from First Seven Cycles Using the PPSQ-50A Gradient System

MALDI-8020: Sequence Analysis by ISD

In Source Decay Sequencing by MALDI-TOF MS

Sequencing by mass spectrometry uses the mass differences between fragment ions to determine the peptide sequence. With in source decay, fragmentation is achieved by destabilizing the analytes by increasing the laser power. The resulting fragments (c-ions typically) represent different peptide lengths from cleavage at the N-Cα bonds along the peptide. Fig. 4 represents the ISD spectrum for BNP.

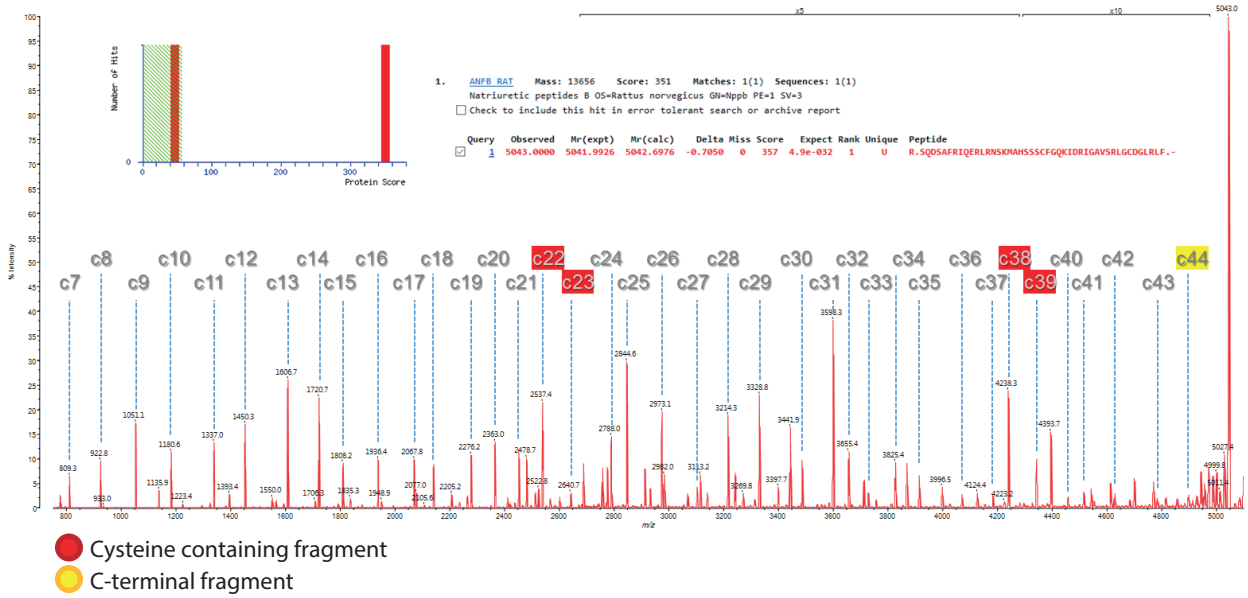

Fig. 4 ISD Spectrum of BNP Using MALDI-8020 with DAN Matrix. MASCOT Identification Shown Inset

Conditions

ISD

- Unit: MALDI-8020 (Linear mode)
- Matrix: 1,5-Diaminonapthalene

Intact Mass

- Unit: MALDI-8020 (Linear mode)
- Matrix: α-cyano-4-hydroxy-cinnamic acid
- Matrix application: iMLayer™

From this data, the resulting sequence can be determined in two ways: database searching or de novo sequencing. Database searching uses the measured masses to compare against a database to determine the peptide sequence. This is the easiest and fastest method, but can be inaccurate or inconclusive as the method depends on the contents of the database. De novo sequencing avoids database usage, but is more labor intensive and prone to user error. Software, such as Mass ++™ can help facilitate this analysis and remove the need for manual data interpretation.

MALDI-8020: Intact Mass Determination

Accurate mass of the intact species can also provide a great deal of information about the peptide or protein. This mass can help give an quick indication of incorrect amino acid composition, possible degradation or the presence of modifications. Accurate and average molecular weight of the intact peptide can be easily determined through proper matrix selection (Table 1). Even with a simple linear configured MALDI-TOF MS, the mass was detected within 20 ppm of the theoretical molecular weight.

Table 1 Theoretical and Measured Masses for BNP

Peptide	Expected Mass (MH+)	Measured Mass (MH+)	Mass Accuracy (ppm)
BNP	5038.6	5038.5	20

Fig. 5 illustrates the detection of the intact mass of BNP using the MALDI-8020.

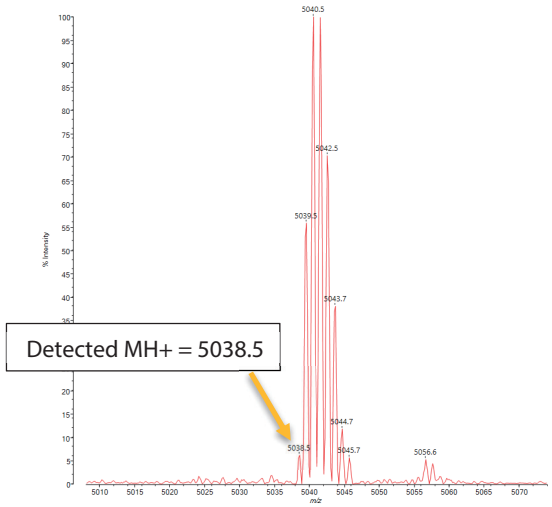

Fig. 5 Intact Mass Spectrum of BNP

Results and Conclusion

MALDI-TOF MS and Edman sequencing both offer great benefit to peptide sequencing as discussed in Table 2. Of all the methods available today, Edman Sequencing remains the best method for determining the actual N-terminus of a protein or peptide. ISD is also a reliable means of obtaining sequence information, but low mass fragments associated with the N-terminus are generally inferred due to the low mass interferences from the matrix. Fig. 6 shows the combined results between the PPSQ and MALDI-8020 for BNP. Whereas using one of these methods can provide a portion of the sequence, using these complementary techniques, complete sequence coverage was obtained.

Fig. 6 Combined Sequence Determination by ISD and Edman Sequencing for BNP

Table 2 Summary Table of Attributes Determined by PPSQ-50 Gradient System and MALDI-8020

Attribute	PPSQ-50 Series	MALDI-8020
N-terminal Sequencing	☑	
Internal, or C-terminal Sequencing		☑
Differentiation of Isobaric Amino Acids	☑	
Avoidance of Databases	☑	
Ease of Data Interpretation (Sequence)	☑	
Ease of Use	☑	☑
Speed of Analysis		☑
Intact Mass Determination		☑

First Edition: Aug. 2019

Shimadzu Corporation
www.shimadzu.com/an/