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Analysis Platform for accurate amino acid sequencing combining with a benchtop MALDI-TOF MS and N-/C-terminal sequencing

Nanami Sakashita[1], Tomoko Kuriki[1], Brian J. Field[2] , Yuzo Yamazaki[1]

1 Shimadzu Corporation. 1, Nishinokyo-Kuwabaracho Nakagyo-ku, Kyoto 604–8511, Japan, 2 Shimadzu Scientific Instruments, Inc., 7102 Riverwood Drive, Columbia, Maryland 21046, U.S.A.

1. Overview

Combining a data from ISD and a complementary one from chemical/enzymatic reaction provides a more complete and accurate peptide sequence.

2. Introduction

Mass spectrometry has become an indispensable tool for researchers looking to sequence peptides. In-source decay (ISD), which is a one of the sequencing techniques performed in MALDI-TOF MS, provides high-throughput sequencing relying on database. Besides a stream of high functionalization, demands for convenient MS instruments is growing to perform the sequencing. Meanwhile, traditional Edman sequencing is still reliable method, because it avoids the use of databases by analyzing retention time of each amino acid from the N-terminus one by one. We will report a peptide sequencing combining ISD using a benchtop liner MALDI-TOF MS and other chemical/enzymatic reaction. The combined sequencing is complementary and sufficient precise to perform a database search, which enables a confirmation of amino acid sequence certainly.

PPSQ-53AG
Gradient

MALDI-8020

Figure 1. A protein sequencer using Edman Degradation (PPSQ-53AG, Shimadzu Corp), and a benchtop liner MALDI-TOF MS (MALDI-8020, Shimadzu Corp.)

Table 1. Feature comparison between Edman sequencing and MALDI-TOF MS

	Edman sequencing	MALDI-TOF MS
N-terminal analysis	*****	*
Removal of blocked N-terminal	Necessary	Not necessary
Internal, or, C-terminal analysis	***	*****
Through-put	*	*****
Existence of modification	*****	***
Detail of modification	**	*****

3. Methods

Peptides including rat BNP-45 was purchased from Peptide Institute, Inc. Mascot server was used for analysis of the data by ISD.
BNP-45 was mixed with dilution series of carboxypeptidase Y (CPY). After incubation, the mixture was spotted onto a sample plate, which was pre-coated with alpha–cyano-4-hydroxycinnamin acid (CHCA) by thin layer method. Peptides were mixed with 1,5-diaminonaphthalene (1,5-DAN), and spotted onto sample plate for ISD. MS experiments in positive ion mode were performed with a benchtop liner MALDI-TOF MS (MALDI-8020, Shimadzu Corp., Japan) at 200 Hz repetition rate of laser.
N-terminal sequencing of peptides was performed with a commercial protein sequencer (PPSQ-50A gradient system, Shimadzu Corp., Japan). Separation column was Wakosil PTH-GR(S-PSQ) and the mobile phases were PTH-amino acids as mobile phase A and PTH-amino acids as mobile phase B. These column and mobile phases were purchased from FUJIFILM Wako Pure Chemical Corporation.

- 45 amino acid residues
- Cyclic peptide including disulfide bond
- A peptide hormone that has vasodilatation and diuretic effect.

Figure 2. Characteristic of brain natriuretic peptide (BNP)

4. Results

4-1. N-terminal sequencing by Edman degradation

Sequencing by Edman degradation was conducted using a protein sequencer after reduction and alkylation of the cysteines. The Edman sequencing based on a high-sensitive gradient HPLC system provided a conclusive N-terminal sequence of the BNP-45. Whereas the advantage of Edman sequencing is avoiding using of database because a sequence is interpreted with reliable retention times of each derivatized amino acid, sequencing modified amino acids including alkylated cysteine is quite difficult. Furthermore, it is unable to analyse N-terminal blocked peptide by the method.

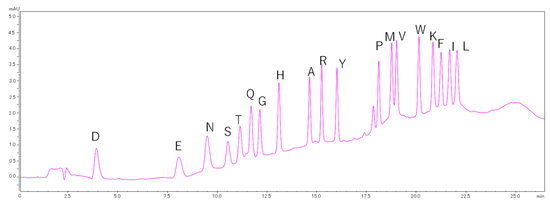


Figure 3. Chromatogram of 500 fmol derivatized amino acid standard

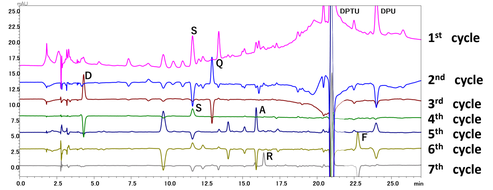


Figure 4. Subtracted chromatograms from 1st cycle to 7th cycle

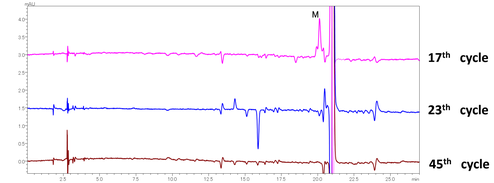
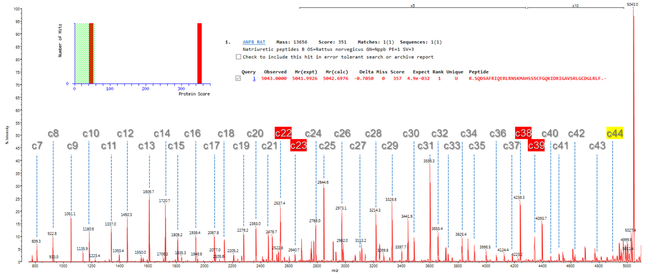


Figure 5. Subtracted chromatograms of cysteine and C-terminus amino acid

4-2. Sequencing by ISD

Since the peptide turns a liner chain due to a reductive effect of 1,5-DAN on the disulfide bond, c-series ions of BNP-45 were obtained in ISD at the wide range from m/z 900 to 5000. Consequently, the BNP-45 was identified with a high confidence in a database search. Notably, two cysteines responsible for the disulfide bond were also clearly identified in ISD. However, it is difficult to analyse N-terminal sequence using the ISD because of interferences at low molecular weight attributable to matrix signals.



Cysteine containing fragment
C-terminal fragment

Figure 6: ISD spectrum of BNP-45 MASCOT identification shown inset.

4-3. C-terminal sequencing by enzymatic reaction

The mass spectra of BNP-45 incubated with CPY are shown in Fig. 7. The C-terminal region of BNP-45 was digested by CPY successfully. As shown in Fig.7, mass gaps between resultant signals were consisted with the C-terminal sequence of BNP-45.

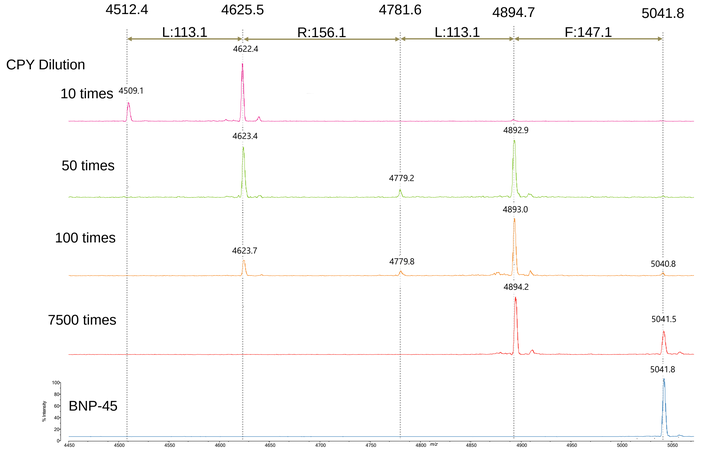


Fig.7 Mass spectra of BNP-45 digested by CPY

5. Conclusions

An amino acid sequence of BNP-45 was identified except N-terminus by ISD and enzymatic reaction using MALDI-TOFMS. On the other hand, the sequence other than C-terminus and cysteine was identified with Edman sequencing. Two complementary sequencings enabled to provide amino acid sequence certainly.

Edman Sequencing Results

H_2N-SQDSAFRIQERLRNSKMAHSSSCFGQKIDRIGAVSRLGCDGLRLF-COOH

MALDI-TOF MS Results

Amino acid identification unique to method
Amino acid identified by both methods

Figure 8: Combined sequence determination by MALDI-TOF MS and Edman Sequencing for BNP-45