Fast Peptide Sequencing without MS/MS? Peptide Primary Structure Elucidation by Mass Spectrometry using Partial Hydrolysis

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Abstract: A straightforward procedure for the primary structure elucidation of peptides is presented. It is found that hydrolysis attacks only the N- and C-ends of peptides leading to partial cleavage of adjacent amino acids. Depending on the peptide, either a single or two overlapping peptide ladders are created which can be analysed using a simple mass spectrometer such as a linear time-of-flight mass spectrometer (TOF-MS) with no MS/MS capabilities.

The procedure is demonstrated on the peptide Melittin, a 26 amino acid single chain peptide. Melittin was partially hydrolysed. After only 1 min of hydrolysis time two peptide ladders were already fully developed and could be analysed. It was found that Melittin is cleaved from both the C- and the N-terminus. About 80% of the primary structure can be determined from the mass spectrum obtained by MALDI-linear TOF-MS.

Advantages of the methodology described are its simplicity and quickness and the fact that there is no need for high-performance mass spectrometers.

Besides its analytical value, the partial hydrolysis procedure can be used for educational purposes such as lab courses for students at Universities of Applied Sciences to introduce concepts of modern peptide structure analysis in combination with mass spectrometry.

Keywords: Education · MALDI-MS · Melittin · Partial hydrolysis · Peptide ladder · Peptide sequence

1. Introduction

One of the first analytical steps in the research fields of genomics or proteomics is the identification of the molecules involved and the elucidation of their structures.

There are several possible strategies for primary sequence analysis such as the N-terminal chemistry of the Edman degradation, enzymatic digestions using peptidases, chemical hydrolysis or MS/MS approaches.

Matrix-assisted laser desorption (MALDI) mass spectrometry is especially well suited for the analysis of complex mixtures, since mainly one molecular ion is generated from every molecular species. Under conventional conditions (e.g. linear TOF-MS), MALDI spectra contain non-abundant fragment ions. However, in conjunction with enzymatic digests or chemical methods such as partial hydrolysis, it is possible to obtain valuable sequence and structural information at three levels as shown in the Table [1].

For peptide structure analysis, traditional hydrolysis is certainly of less importance due to the fact that non-specific cleavage has to be expected. This would generate complex patterns of all internal peptides. However, Knierman et al. [2] have shown that partial hydrolysis can produce peptide fragments which are rapidly cleaved at the peptide bonds from either the N- or C-terminus (depending on the peptide) but much more slowly from inside the peptide chain. Consequently, the fragments of a partial hydrolysis form so-called ‘peptide ladders’ [3] in a mass spectrum.

The sequence of the amino acids can be directly read in the correct order using the mass differences between adjacent fragment peaks in the mass spectrum of the peptide.

Table. Information obtainable on peptides and proteins by mass spectrometry

- Molecular masses of the intact peptide protein
- Peptide maps (typical mixtures of peptide/protein fragments after enzymatic or chemical degradation)
- Sequence analysis (through chemical means or MS/MS approaches)
the 'peptide ladder' as shown in Fig. 1. The measured mass difference refers to the released amino acid except for Leu/Ile which have identical masses and a few others such as Gln/Lys with too small mass differences to be distinguished in a mass spectrometers with medium resolution and mass accuracy.

In short: The remaining fragments after the stepwise release of the amino acids from the termini are collected and analysed instead of the released amino acids.

2. Results and Discussion

The analysed peptide was Melittin, the main component of honey bee venom, which consists of 26-amino acids in a single chain. It is a hydrophobic peptide with the amino acid sequence GIGAVLKVLTGLPALISWIRKQRQ-NH₂ and a molecular mass of 2846.5 Da.

This peptide was partially hydrolysed. The hydrolysis was carried out in 3M HCl at 110 °C. After certain time intervals aliquots were withdrawn and directly analysed (Fig. 2) on a Applied Biosystems Voyager DE MALDI-mass spectrometer with a linear TOF-MS and delayed extraction.

Fig. 1. Principle of the amino acid determination by a stepwise degradation of the peptide chain and subsequent analysis of the resulting 'peptide ladder' by mass spectrometry.

Fig. 2. Positive ion MALDI mass spectra of the evolving 'peptide ladders'. The dashed line indicates the position of the ion signal of the intact protonated Melittin molecule at 2847.5 Da. Matrix: 2,5-dihydroxybenzoic acid.
Since no more 'useful' peptide ladder fragments were produced after 60 sec and to prevent fragments created by internal bond cleavage, the hydrolysis reaction was stopped. The mass spectrum of the last aliquot together with the identified amino acids are shown in Fig. 3.

All ion signals detected in the mass spectrum shown in Fig. 3 originate from two overlapping 'peptide ladders' of C- or N-terminally truncated Melittin peptides. No random cleavages at internal peptide bonds were detected after 1 min. If the peptide is truncated from only one end, the interpretation is straightforward. Melittin, however, is truncated simultaneously from both the C- as well as the N-terminus.

Nevertheless, it was possible to determine about 80% of the amino acid structure of Melittin by comparing the mass differences between adjacent peaks in the mass spectrum with the masses of the naturally occurring amino acids. The identified amino acids of Melittin as well as their positions are indicated in Fig. 3. By mixing different aliquots and analysing the corresponding mass spectrum the percentage of coverage of the peptide amino acid sequence can be expected to be higher (not shown).

The combination of the simple and fast hydrolysis procedure with rapid mass spectrometric analysis can help to obtain enough information to elucidate peptide structures. The interpretation is difficult if two overlapping ladders are present. A computer program is under development to automate the relatively intricate process of identifying the correct amino acids in such a mass spectrum.

In combination with databases it is possible to use the determined sequences to identify the peptide [4] or to confirm its structure. This procedure could further be helpful to accelerate the sequencing of unknown peptides.

3. Impact for Education

As a welcome side effect, the described procedure is especially well suited for educational purposes such as (in our case) a lab course in instrumental analysis at the University of Applied Sciences at Burgdorf. It is fast, straightforward and the analysis can be performed on a 'simple' mass spectrometer such as a linear TOF-MS which can be found nowadays in chemical laboratories.

The analysis of the primary structure of peptides confronts students with peptide chemistry, biopolymer analysis and mass spectrometry. Peptide samples can be identified using the discovered sequences and protein sequence database-searching on a Proteomics server such as Swiss-Prot [5].

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[1] C. Fenselau, Anal. Chem. 1997, 69, 661A.
[2] M.D. Knierman, J.E. Coligan, K.C. Parker, Rapid Commun. Mass Spectrom. 1994, 8, 1007.
[3] B.T. Chait, R. Wang, R.C. Beavis, S.B.H. Kent, Science 1993, 262, 89.
[4] M. Mann, P. Højrup, P. Roepstorff, Biol. Mass Spectrom. 1993, 22, 338.
[5] www.expasy.ch