Identification of Peptides from Enzymatic Digestion of Human Skin Elastin by Nanoelectrospray Tandem Mass Spectrometry

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1. Overview

Background
- Detailed knowledge about the primary structure and possible post-translational modifications of human skin elastin is needed to understand the biochemical basis of pathological disorders, in which the mechanical and elastic properties of tissues are altered.

Objectives
- Characterization of peptides from proteolytic digestion for the investigation of the primary structure
- Determination of the existence and extent of proline hydroxylation

Realization
- Digestion with the three low-specific proteases thermolysin, elastase, and pepsin
- Analysis by nanoelectrospray tandem mass spectrometry on a quadrupole time-of-flight instrument
- Sequence determination by database matching and a combination of de novo sequencing with database searching

2. Introduction

Elastin is an important constituent of elastic fibers, which play a key role in the elasticity of different tissues, for example in skin, bronchi, blood vessels, and ligaments [1]. Pathological disorders, such as solar elastosis, emphysema, aneurysms, and atherosclerosis, in which the mechanical properties of tissues are altered, are associated with abnormalities in elastic fibers. The investigation of biochemical processes underlying such disorders requires information about the primary structure of elastin, but the extreme insolubility makes structure determination challenging. However, the analysis of elastin digests is a suitable strategy to gain insights into the molecular structure of the protein itself. Therefore, in this work, we determined the sequences of peptides resulting from digestion of human skin elastin with three low-specific proteases.

3. Methods

Elastin extracted from human skin was digested with elastase, thermolysin or pepsin. After desalting, the digests were analyzed by means of tandem mass spectrometry on a quadrupole time-of-flight mass spectrometer (Micromass Q-TOF-2) equipped with a nanoelectrospray (nano-ESI) Z-Spray source.

The mass spectrometric raw data were processed using MassLynx with MaxEnt3. Peptide sequencing was carried out by database searching (Mascot, Matrix Science) and de novo sequencing with combined protein database searching (PEAKS, Bioinformatics Solutions). Besides MSDB and SWISS-PROT a home-made protein database containing elastin sequences with its splice variants was used. The sequencing was performed with regard to a varied hydroxylation of proline.

4. Results

Since the ionization efficiency in nano-ESI is significantly higher than with conventional electrospray, even complex mixtures such as the investigated digests can be analyzed directly without the need of chromatographic separation. Whereas elastin is resistant to site-specific enzymes such as trypsin and chymotrypsin, the hydrolysis by the proteases applied in this study was very effective. About 90 peptides having 2-26 amino acids were unequivocally identified from the three different digests. Without consideration of numerous residues with multiple potential positions in the precursor protein, the sequence coverage was 51% (see Fig. 1). This percentage is in reference to the elastin sequence on the PIR database entry A32707 (EAHU) excluding the exons 22 and 26A, which are known to be missing in human skin elastin [2]. Numerous prolines were found to exist in a hydroxylated and non-hydroxylated state in parallel (Fig. 2). De novo sequencing enabled the unequivocal assignment of hydroxylation sites. It was also an indispensable tool for the verification of ambiguous Mascot results.

5. Conclusions

- The proteases were found to be suitable for the hydrolysis of insoluble elastin that can not be digested with site-specific enzymes.
- The existence of prolines in a hydroxylated and non-hydroxylated state in parallel indicates a partial hydroxylation of the tropoelastin molecule.
- The described approach allowed the study of the primary structure of elastin extracted from tissue samples and the analysis of pathological modifications.

References