Identification of amino acid sequences for some peptides resulting from enzymatic digestion of human skin elastin using LC/MS/MS

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Introduction

Elastin is an important connective tissue protein that provides elasticity to organs, such as skin, lung, aorta, and ligaments. Characterization of elastin could be useful in helping to understand the biochemical basis of several pathological conditions, including solar elastosis, emphysema, aneurysms and atherosclerosis, in which the mechanical and elastic properties of tissues are altered. However, due to its extreme insolubility, such studies are mainly focused on complete hydrolysis of the protein in strong acidic environment and analysis of the resulting characteristic cross-linked amino acids, desmosine and its isomer isodesmosine [1-4]. This approach has been challenged, among other things, by the incomplete separation of the amino acids with the analytical methods employed to date and the partial information that can be obtained only from the cross-linked amino acids about the entire elastin molecule. Extraction of elastin from healthy and diseased tissues and comparison of the molecular structures of the peptides resulting from enzymatic digestion of the protein could give a better image of the biochemical changes that occur due to the mentioned pathological conditions. Thus, in this work, the amino acid sequences of some of the peptides formed as a result of enzymatic digestion of elastin was determined with the help of LC/MS/MS and database search tools.

Experimental

Materials

Elastin extracted from human skin and elastase from porcine pancreas were purchased from Elastin Products Company (Owensville, Missouri, USA).

Thermotase from Thermoactinomyces vulgaris was kindly offered by Dr. Ulrich Rothe, Institute of Physiological Chemistry, Martin Luther University Halle-Wittenberg, Germany.

HPLC grade acetonitrile was obtained from J. T. Baker (Deventer, The Netherlands) and formic acid from Merck (Darmstadt, Germany).

Methods

Enzymatic digestion of human skin elastin

Elastin (1mg/ml in 1mM Tris buffer, pH 8.5) was digested with elastase or thermotase for 24 h at 37°C. Enzyme-substrate ratio was 1:50.

LC-ESI mass spectrometry

The elastin digests were diluted to 0.5 mg/ml in 1mM Tris buffer, pH 8.5 and analysed by LC-ESI-MS using a Finnigan LCQ ion trap mass spectrometer (ThermoFinnigan, San Jose, CA, USA) coupled with electrospray interface to a Spectra System P 4000 HPLC pump equipped with an autosampler AS 3000.

10 μl of each sample solution were loaded onto a Nucleosil® 120-5 C18 column (125 x 2 mm i.d., Macherey-Nagel, Duren, Germany) and peptides eluted using a linear gradient: 5-60% of acetonitrile in distilled water, both containing 0.1% of formic acid, over 60 min. The column was maintained at 30°C and flow rate was 0.2 ml/min.

Peptides of interest were selected manually based on the relative intensity of their m/z peaks for further tandem MS experiments using collision induced dissociation (CID).

Peptide identification

The fragment ion spectra of ESI-MS/MS obtained were processed using Mascot Distiller, which reduces raw mass spectrometry data to high quality peak lists, and analyzed searching the Mass Spectrometry Protein Sequence Database (MSDB) with Mascot (Matrix Science Ltd., London, UK) [5]. The searches were taxonomically restricted to human.

Results

LC/MS chromatograms of the digests of human skin elastin with elastase or thermotase are shown in Figure 1.

Some peptides were selected based on the relative abundance of their [M+H]+ ions, MS/MS fragmentation was performed on these ions and database searching was done using the fragment ion spectra of each of the ions (Fig. 2).

The sequences of 19 and 31 peptides from the elastase and thermotase digests, respectively, were identified (Tables 1 and 2).

The sequences of these peptides are shown on the tropoelastin sequence (Fig. 3). Sequences highlighted with the same color share the same sequence partially or wholly.

A high degree of similarity in substrate specificity of the two enzymes was also observed. Both enzymes acted at the C-terminals of the amino acids Gly, Ala, Val, Leu and Phe. The effect of thermotase on Ala and Pro is different than that of elastase. Both enzymes cleaved Ala, in positions giving rise to smaller peptides, and acted at the C-terminals of the amino acids Gly, Ala, Val, Leu and Phe. The effect of thermotase on Ala seems to be higher than that of elastase and the former also cleaved at Glu.

References