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In vitro cross-linking of elastin peptides and molecular characterization of the resultant biomaterials

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ABSTRACT

Background: Elastin is a vital protein and the major component of elastic fibers which provides resilience to many vertebrate tissues. Elastin's structure and function are influenced by extensive cross-linking, however, the cross-linking pattern is still unknown.
Methods: Small peptides containing reactive allysine residues based on sequences of cross-linking domains of human elastin were incubated in vitro to form cross-links characteristic of mature elastin. The resultant insoluble polymeric biomaterials were studied by scanning electron microscopy. Both, the supernatants of the samples and the insoluble polymers, after digestion with pancreatic elastase or trypsin, were furthermore comprehensively characterized on the molecular level using MALDI-TOF/TOF mass spectrometry.
Results: MS^2 data was used to develop the software PolyLinX, which is able to sequence not only linear and bifunctionally cross-linked peptides, but for the first time also tri- and tetrafunctionally cross-linked species. Thus, it was possible to identify intra- and intermolecular cross-links including allysine aldols, dehydrolysinonorleucines and dehydromerodesmosines. The formation of the tetrafunctional cross-link desmosine or isodesmosine was unexpected, however, could be confirmed by tandem mass spectrometry and molecular dynamics simulations.
Conclusions: The study demonstrated that it is possible to produce biopolymers containing polyfunctional cross-links characteristic of mature elastin from small elastin peptides. MALDI-TOF/TOF mass spectrometry and the newly developed software PolyLinX proved suitable for sequencing of native cross-links in proteolytic digests of elastin-like biomaterials.
General significance: The study provides important insight into the formation of native elastin cross-links and represents a considerable step towards the characterization of the complex cross-linking pattern of mature elastin.

1. Introduction

Elastin is one of the most important proteins of the extracellular matrix of vertebrates. As the core protein of elastic fibers, elastin provides elasticity and resilience to tissues including aorta, lung, skin, ligaments, tendon and cartilage and is, thus, critical for their long-term function. It is secreted in the form of its monomeric precursor tropoelastin that consists of alternating highly hydrophobic and more hydrophilic K-containing domains, of which the more hydrophobic regions are responsible for self-aggregation and tensile properties of elastin and the latter are involved in cross-linking to form an insoluble and durable polymer highly resistant to proteolytic degradation [1–3].

While it is recognized that elastic fiber networks in different tissues are organized differently, e.g. concentric fenestrated lamellae in the medial layer of the aorta or honeycomb-like structures in elastic cartilage, and that their function is strongly influenced by their composition, organization and architecture, virtually nothing is known about the cross-linking pattern in different human tissues and the exact domains of tropoelastin molecules that are involved in cross-linking. The only report on the exact location of a cross-link in elastin has

Abbreviations: AA, allysine aldol; ACN, acetonitrile; CHCA, α-cyano-4-hydroxycinnamic acid; CID, collision-induced dissociation; DES, desmosine; Δ-LNL, dehydrolysinonorleucine; Δ-MD, dehydromerodesmosine; EP, elastin-like polypeptide; FA, formic acid; HPLC, high performance liquid chromatography; IDES, isodesmosine; k, allysine residue; LID, laser-induced dissociation; LOX, lysyl oxidase; MALDI, matrix-assisted laser desorption/ionization; MD, molecular dynamics; MS, mass spectrometry; PDF, probability density function; PQQ, pyrroloquinoline quinone; PyBOP, benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate; RMSD, root mean square deviation; SEM, scanning electron microscopy; SVM, support vector machine; TFA, trifluoroacetic acid; Tris, 2-amino-2(hydroxymethyl)-1,3-propanediol

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been made by Brown-Augsburger et al. who used Edman degradation to identify a cross-linked structure in porcine elastin formed through the association of three tropoelastin chains of domains 10, 19 and 25 [4]. With respect to the formation of elastic fibers, it is known that microfibrils act as a scaffold for the deposition of tropoelastin after its secretion into the extracellular space where coacervation and structural alignment occur prior to cross-linking. Initially, allysine (α-aminoadipic acid-δ-semialdehyde) is produced through oxidative deamination of the ε-amino group of a K residue by the enzyme lysyl oxidase (LOX) or LOX-like proteins. Intra- and intermolecular cross-links are subsequently formed either by non-enzymatic condensation of two allysine residues via aldol condensation which produces allysine aldol (AA) or by reaction of an allysine residue with the ε-amino group of another lysine residue via Schiff base reaction which creates dehydrolysinonorleucine (Δ-LNL). Such reducible cross-links then further condense to form the stable and non-reducible trifunctional cross-links merodesmosine and cyclopentenosine, the tetrafunctional cross-links desmosine (DES) and isodesmosine (IDES) as well as pentafunctional cross-links such as allodesmosine and pentasine [1–3,5–12]. The structures and formation pathways of prominent elastin cross-links are shown in Fig. 1.

Owing to its unique structure, elastin does not undergo significant turnover in healthy tissues [13]. Pathological conditions and diseases, including emphysema, chronic obstructive pulmonary disease, atherosclerosis and actinic elastosis (photoaging), however, have been identified to be associated with changes in the structure, distribution and abundance of elastin which often involves the destruction of elastic fibers [2,3,14]. Thus, elucidating the molecular-level structure of human elastin including the cross-linking pattern in different tissues and comparing the structures of elastin from healthy and diseased tissues would help to better understand the biomechanical properties of elastin as well as elastic-tissue diseases and their functional consequences.

In recent years, mass spectrometry (MS) has become important for the determination of low resolution 3D structures of proteins using a technique called MS3D (MS in three dimensions) [15]. In MS3D experiments, proteins are chemically cross-linked, digested by site-specific proteases and the resultant cross-linked peptides are identified using bioinformatics methods and used to derive inter-atomic distance constraints. Available software solutions such as xQuest [16], VIRTUALMSLAB [17], CLPM [18] or pLink [19], however, mainly deal with chemically introduced and only bifunctional cross-links. Since elastin shows unique tri-, tetra- and pentafunctional cross-links in addition to bifunctional cross-links, there is a need for the development of new bioinformatics tools that are able to deal with MS data of such peptides. However, it is very challenging analytically as well as computationally to identify cross-linked peptides from elastin since the protein is very hydrophobic and has a highly repetitive sequence, with 78% of human tropoelastin (isoform 2) composed just of the four amino acids G, A, V and P. Hence, peptides released during proteolysis often have similar or even identical masses and show similar product ion spectra when subjected to tandem MS (MS/MS). Moreover, alternative splicing, the hydroxylation of P and especially the many types of cross-links together with the high number of the highly similar cross-linking domains result in a huge number of possible combinations for cross-links. This is reflected in a tremendous number of theoretical peptides in an enzymatic digest of elastin which is further multiplied since proteases with broad specificities have to be used to solubilize elastin. Further challenges arise from the fact that the cross-linking of two tropoelastin domains can take place between chains that are oriented either in a parallel or antiparallel manner. Moreover, the fragmentation behavior of such cross-linked species is different from the typical backbone fragmentation of linear peptides and has not yet been investigated. For instance, in dissociation experiments some fragments can remain cross-linked whereas others do not. Overall, based on product ion spectra of cross-linked peptides derived from mature elastin it is hard and in some cases probably impossible to assign the peptides to unique positions in the involved monomeric sequences.

Therefore, in this study small deca- and undecapeptides based on cross-linking domains of human elastin were used as model substrates to produce defined cross-linked species characteristic of mature elastin. Based on these cross-linking domain peptides, mass spectrometric and bioinformatics methods designed for the identification of cross-linked species were developed successfully. This work represents an important

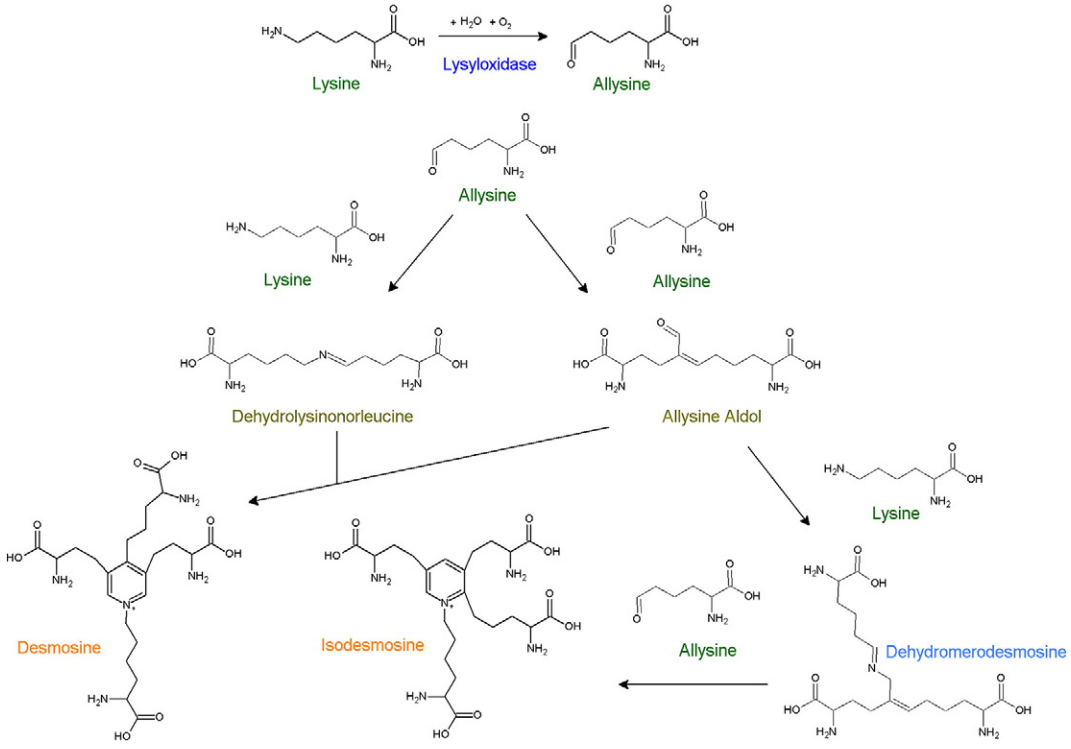

Fig. 1. Formation of bi-, tri- and tetrafunctional cross-links in elastin (adapted from [3]).