Characterizing cross-links of the matrix protein elastin using MALDI-TOF/TOF-mass spectrometry and bioinformatics approaches

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Elastin

- Cross-linked structure
 protein
- Very hydrophobic, insoluble in any solvent
- Soluble precursor tropoelastin (60 kDa – 70 kDa)
- Tropoelastin exists in various isoforms (alternative splicing)
- Associated with various severe diseases (destruction of elastic fibers, release of bioactive peptides)



Tropoelastin isoform 9





Cross-linking in elastin



Hydrophobicity/Hydrophilicity plot of human tropoelastin (Swiss-Prot accession number P15502, Isoform 9) calculated by the method of Hopp and Woods (1981).

Cross-linking in elastin



Analytical challenges

- Time-consuming proteolysis since elastin is very resistant towards enzymatic degradation
- Peptides mainly hydrophobic
- Many peptides with equal or same masses
- Many repetitive regions and, thus, high similarity between fragmentation patterns
- Cross-linked peptides cannot be sequenced with available software tools

Mass	search result	s							. 🗆	×
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	711,3915	[1]	Ο	573-	581	AA	AGLGAG	IPG	LG	
	711,3915	[1]	0	548-	556	PG	IGPGGV.	AAA	AK	
	711,3915	[1]	0	535-	543	AP	GVGVAP	GVG	VA	
	711,3915	[1]	0	529-	537	AP	GVGVAP	GVG	VA	
	711,3915	[1]	0	517-	525	\mathtt{AP}	GVGVAP	GVG	LA	
	711,3915	[1]	0	511-	519	\mathtt{AP}	GVGVAP	GVG	VA	
	711,3915	[1]	0	505-	513	$\boldsymbol{V}\boldsymbol{P}$	GVGVAP	GVG	VA	
	711,3915	[1]	0	672-	680	$\mathbb{A}\mathbb{L}$	GGVGIP	GGV	VG	
	711,3915	[1]	0	680-	688	GG	VVGAGP.	AAA	AA	
	711,3915	[1]	0	408-	416	\mathbf{GF}	GVGVGG	IPG	VA	
	711,3915	[1]	0	715-	723	GV	GGLGVP	GVG	GL	
	711,3915	[1]	0	713-	721	GL	GVGGLG	J PG	VG	
	711,3915	[1]	0	719-	727	$\mathbf{L}\mathbf{G}$	VPGVGG	LGG	IP	
	711,3915	[1]	0	718-	726	GL	GVPGVG	GLG	GI	
	711,3915	[1]	0	716-	724	VG	GLGVPG	IGG	LG	
	711,3915	[1]	0	277-	285	PG	VGGAGV	PGV	PG	
	711,3915	[1]	0	268-	276	FG	AGAAGVI	LPG	VG	
	711,3915	[1]	0	356-	364	\mathbf{VP}	GAGIPG.	AAV	PG	
	711,3915	[1]	0	338-	346	\mathbf{VP}	GAGVPG	JGV	PG	
	711,3915	[1]	0	267-	275	KF	GAGAAG	ΛΓΡ	GV	
	711,3915	[1]	0	332-	340	GP	GVVGVP	GAG	VP	
	711,3915	[1]	0	318-	326	ΥG	AAAGLVI	PGG	PG	
	711,3915	[1]	0	317-	325	KΥ	GAAAGLY	JPG	GP	
	711,3915	[1]	0	114-	122	$\mathbf{L}\mathbf{G}$	GVPGVG	GLG	vs	
	711,3915	[1]	0	113-	121	GL	GGVPGV	GGL	GV	
	711,3915	[1]	0	112-	120	ÅG	LGGVPG	IGG	LG	
	711,3915	[1]	0	111-	119	GA	GLGGVP	GVG	GL	
	711,3915	[1]	0	67-	75	ΡV	PGGLAG.	AGL	GA	
	711,3915	[1]	0	50-	58	$\mathbf{L}\mathbf{G}$	ALGGGA	LGP	GG	
	711,3915	[1]	0	44-	52	FΥ	PGAGLG.	ALG	GG	
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Analytical methods

- Mass spectrometry
- Scanning electron microscopy



Ultraflex III (Bruker Daltonics)



Q-TOF-2 (Waters Micromass)



Voyager DE Pro (ABSciex)



Synapt G2 HDMS (Waters)



LTQ Orbitrap XL (Thermo)



4800 MALDI TOF/TOF Analyzer (ABSciex)

Experimental design

1. Short elastin peptides

3. Tropoelastin-

Cross-linked using Aspergillus nidulans amine oxidase



2. Elastin domains EP20-24-24 Cross-linked using pyrroloquinoline quinone









Cross-linking of short elastin peptides

Possible types of cross-links





Identified cross-linked species

Involved peptides	Type of cross-link	Mass (Da)
P1	Dehydrolysinonorleucine	865.466
P2	Dehydrolysinonorleucine	1079.598
P1, P1	Dehydrolysinonorleucine	1748.942
P1, P1	Allysine aldol	1748.942
P1, P1	Dehydromerodesmosine	1730.931
P2, P2	Dehydrolysinonorleucine	2177.206
P2, P2	Allysine aldol	2177.206
P2, P2	Dehydromerodesmosine	2159.195
P1, P1, P1	Desmosine	2595.389
P2, P2, P2	Desmosine	3237.784

Bifunctional cross-link Trifunctional cross-link Tetrafunctional cross-link



Molecular dynamics simulation



Scoring

Potential b and y ions resulting from the fragmentation of a peptide cross-linked via dehydrolysinonorleucine.



Labeled TOF/TOF fragment spectrum of cross-linked species containing dehydrolysinonorleucine with a precursor mass of 1749.94 Da.

Cross-linking of elastin domains

Elastin domains

EP 20-24 (Length: 117 amino acids, MW: 9,916.48 Da)

FPGFGVGVGGIPGVAGVPGVGGVPGVGGVPGVGIPEAQAAAAAKAAKYGVGT PAAAAAKAAQFGLVPGVGVAPGVGVAPGVGVAPGVGLAPGVGVAPGVG VAPGVGVAPAIGP

Exons tropoelastin: 20-21-23-24

EP 20-24-24 (Length: 199 amino acids, MW: 16,894.36 Da)

FPGFGVGVGGIPGVAGVPGVGGVPGVGGVPGVGIPEAQAAAAAKAAKYGVGT PAAAAAKAAAKAAQFGLVPGVGVAPGVGVAPGVGVAPGVGLAPGVGVAPGVG VAPGVGVAPAIGPEAQAAAAAKAAKYGVGTPAAAAAKAAAKAAQFGLVPGVGV APGVGVAPGVGVAPGVGLAPGVGVAPGVGVAPGVGVAPAIGP

Exons tropoelastin: 20-21-23-24-21-23-24

Workflow



Cross-linked EP 20-24-24

Free allysine residues

- 1 FPGFGVGVGGIPGVAGVPGVGGVPGVGGVPGVGIPEAQAAAAAKAAkYGVGTPAAAAAkA
- 61 AAkaaqfglvpgvgvapgvgvapgvgvapgvglapgvgvapgvgvapgvgvapgvgvapaigpeaq
- 121 AAAAAKAAkYGVGTPAAAAAkAAAkAAQFGLVPGVGVAPGVGVAPGVGVAPGVGLAPGVG
- 181 VAPGVGVAPGVGVAPAIGP

identified from digests of EP 20-24-24 with pancreatic elastase, trypsin and chymotrypsin

6 of 8 lysine residues were found to be oxidized, however, non cross-linked

Low degree of cross-linking

Intramolecular cross-link







OH

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ΗŃ

CH₃

OH

CH₃

Tetrapropionylated desmosine

Degree of DES	Monoisotopic Mass
propionylation	[Da]
0	526.29
1	582.31
2	638.34
3	694.37
4	750.39

Tetrafunctional desmosine



MALDI TOF/TOF tandem mass spectra of propionylated desmosine ([M+H] 750.39).

Cross-linked tropoelastin

Free allysine residues

1 MAGI, TAAAPRPGVI, I, I, I, I, I, STI, HPSRPGGVPGATPGGVPGGVFYPGAGI, GAI, GGGAI, GPGG 61 **k**PLKPVPGGLAGAGLGAGLGAFPAVTFPGALVPGGVADAAAAYKAAKAGAGLGGVPGVGG 121 LGVSAGAVVPQPGAGVKPGkVPGVGLPGVYPGGVLPGARFPGVGVLPGVPTGAGVkPkAP 181 GVGGAFAGIPGVGPFGGPQPGVPLGYPIKAPkLPGGYGLPYTTGkLPYGYGPGGVAGAAG 241 kagyptgtgvgpoaaaaaakkaaakfgagaagvlpgvggagvpgvpgaipgiggiagvgt 301 PAAAAAAAAAAAAKAAKYGAAAGLVPGGPGFGPGVVGVPGAGVPGVGVPGAGIPVVPGAGIP 361 GAAVPGVVSPEAAAKAAAKAAKYGARPGVGVGGIPTYGVGAGGFPGFGVGVGGIPGVAGV 421 PGVGGVPGVGGVPGVGISPEAQAAAAAKAAKYGVGTPAAAAAkAAAAGFGLVPGVGVA 481 PGVGVAPGVGVAPGVGLAPGVGVAPGVGVAPGVGVAPGIGPGGVAAAAkSAAKVAAkAOL 541 RAAAGLGAGIPGLGVGVGVPGLGVGAGVPGLGVGAGVPGFGAVPGALAAAKAAKYGAAVP 601 **GVLGGLGALGGVGIPGGVVGAGPAAAAAAAAAAAAAAOFGLVGAAGLGGLGVGGLGVPGV** 661 GGLGGIPPAAAAKAAkYGAAGLGGVLGGAGQFPLGGVAARPGFGLSPIFPGGACLGkACG 721 RKRK

identified in digests of pancreatic elastase, trypsin and chymotrypsin, based on isoform 2 of tropoelastin

13 of 35 allysine residues were found to be deaminated

Intramolecular cross-link

Allysine aldol



100)]	ASVLR	Q	b ₂ y ₁	01410	b ₃	y ₂			y ₃	y	4					a₅ ∶	b ₈	t	9 ₉		b ₁₀	y₀ ∶		b ₁₁ y ₁₀		У ₁₁	[M+H]⁺ 	1.2E+4
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Molecular dynamics simulations



Peptide	Minimal distance [Å]	Maximal distance [Å]	Average distance ± SD [Å]
AAKVAAKAQ	5.2	21.5	15.7 ± 0.3
AA <mark>k</mark> VAA <mark>k</mark> AQ	3.4	18.2	10.3 ± 0.2

Enrichment of cross-linked species



Summary and outlook

- First attempt to elucidate the structure of elastin using mass spectrometry and bioinformatics methods based on model systems of different complexity
 - Cross-linked elastin peptides
 - Cross-linked elastin domains
 - Cross-linked tropoelastin
- Based on fragment spectra of defined cross-linked species, it was possible to develop a software which is able to classify and sequence not only bifunctionally but also tri- and tetrafunctionally cross-linked species
- Further analysis aims to get insights into cross-linked species from enzymatic digests of mature elastin using similar approaches

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