Molekulare Einblicke in die Alterung elastischer Fasern

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Core protein of elastic fibers \rightarrow Elastin

- Very hydrophobic and highly cross-linked fiber protein, insoluble in any solvent
- Soluble precursor: tropoelastin (60 kDa – 70 kDa), undergoes extensive alternative splicing
- High reversible extensibility
- Half-life: ~70 years



Tropoelastin

🕞 [P1550	02-2] P15502	-2 (ELN, HUMA	м)						_ 🗆 🛛
62623,5	51 Da Mo.	75.03 Da	[229]	15 🏭 🗍 🛛	i • a	🕔 Hydrogen 🛛 P	ree acid 🔾 🖡	dose ⊆lose	
1	M <mark>AGL</mark> T	AAA <mark>P</mark> R <mark>PG</mark>	VLLL	LSI <mark>L</mark> HP	SR <mark>PGG</mark>	VPGAIPC	GVPGG,	/FY <mark>PG</mark> A	GLG <mark>A</mark>
51	LGGGA	LGPGG <mark>K</mark> F	PLK PVF	GGL <mark>AG</mark> A	GLG <mark>A</mark> GI	LG <mark>A</mark> F <mark>PA</mark> V	/TF <mark>PG</mark> A,I	LVPGGV	ADAA,
101	AA Y <mark>K</mark> A/	aka <mark>ga</mark> gi	GGVPG	VGGLGV	SAGAV	VP,QPG <mark>A</mark> (GV <mark>K</mark> PG <mark>K</mark> I	/PGVGL	PGV Y,
151	PGGVLI	P <mark>GA</mark> RF <mark>P</mark> G	VGVLF	GVP,TG <mark>A</mark>	GV <mark>K</mark> PK	APGVGG <mark>7</mark>	AF <mark>A</mark> GIP(GVGPFG	GPQP,
201	GVPLG'	Y <mark>PIKA</mark> PK	LPGG Y	GLP YTT	G <mark>K</mark> LPY(G Y GPGG V	/A <mark>G</mark> AA <mark>G</mark>	(<mark>AG</mark> YPT	GTGV,
251	GPQAA/	AAAAAKA	AAK F <mark>G</mark>	A <mark>G</mark> AAGV	LPGVG	G <mark>A</mark> GVPGV	/PG <mark>A</mark> IP	JI <mark>GG</mark> I <mark>A</mark>	GVGT,
301	PAAAA2	1AAAAA	AAK Y <mark>G</mark>	AAA <mark>GLV</mark>	PGGPG]	F <mark>G,PGVV(</mark>	GVPG <mark>A</mark> G,V	/PGVGV	PG <mark>A</mark> G
351	I <mark>PVVP(</mark>	GA <mark>GIPG</mark> A	AVPGV	VS <mark>P</mark> EAA	A <mark>K</mark> AAA	K <mark>aa</mark> k y <mark>g</mark> 2	AR <mark>PGVG</mark> V	/GGIPT	Y <mark>GVG</mark>
401	AGG F P (G <mark>FGVGVG</mark>	GIPGV	AGV,PSV	GGVPG	VGGVPGV	/GISPE/	AQAAAA	A <mark>R</mark> AA,
451	K Y <mark>GVG</mark> '	Г <mark>Р</mark> ААААА	K <mark>AAA</mark> K	<mark>AA</mark> QF <mark>GL</mark>	VPGVG	V <mark>A</mark> PGVGV	/ <mark>A</mark> PGVG	/APGVG	L <mark>A</mark> PG,
501	VGVAP(GVGV <mark>A</mark> PG	VGVAF	GI <mark>G</mark> PGG	VAAAA	K S <mark>AA</mark> K V	AAK <mark>aql</mark> i	R <mark>AAA</mark> GL	<mark>GA</mark> GI,
551	PGLGV(GVGVPGI	.GVG <mark>A</mark> G	VPGLGV	G <mark>A</mark> GVP	GF <mark>GA</mark> VPO	JA <mark>L</mark> AAA	(<mark>AA</mark> K Y <mark>G</mark>	AA <mark>VP</mark>
601	GVLGG]	lg <mark>a</mark> lggv	'GIPGG	VVG <mark>A</mark> GP	AAAAA	AAKAAA	<mark>AA</mark> QF <mark>G</mark> J	LVGAAG	LGGL
651	GVGGL(<u>SVPGVGG</u>	LGGIF	PAAAA K	AAK Y <mark>G</mark> i	AA <mark>GLGG</mark>	/LGG <mark>A</mark> G,	QF <mark>PLGG</mark>	VAAR,
701	PGFGL	3 <mark>P</mark> IF <mark>PGG</mark>	<mark>A</mark> CLG	AC <mark>G</mark> RK R	K				







Structure of elastic fibers









Elastin - a mere structure protein?



Elastases (serine proteases, matrix metalloproteinases)

Cross-linked elastin

Biological effects of matrikines

- Expression of MT-MMP-1, MMP-1 and MMP-2
- Chemotaxis
- Proliferation
- Induction of apoptosis
- Induction of angiogenesis
- Vasorelaxation and NO liberation
- Osteogenesis
- Th-1 polarization



Cell types: fibroblasts, endothelial cells, lymphocytes, monocytes, smooth muscle cells

Isolation of elastin



Isolation of elastin

→ Intact elastin free from contaminants and remnants of the ECM



Skin biopsy





9

One week

Morphological characterization



Human skin elastin isolated after Starcher and Galione



Human skin elastin isolated by a less destructive method

Molecular-level characterization

- Degradation of isolated elastin by porcine pancreas elastase
- Qualitative analysis by nanoHPLC-MS/MS and nanoLC/MALDI-TOF/TOF

Identified precursors	InChorus Score	Identified precursors	InChorus Score
Tropoelastin	99 %	Tropoelastin	99 %
Collagen Type I α-1	99 %		
Collagen Type I α-2	99 %		
Collagen Type III	99 %		



S. Taddese, M. C. Jung, C. Ihling, A. Heinz, R. H. H. Neubert, and C. E. H. Schmelzer, MMP-12 catalytic domain recognizes and cleaves at multiple sites in human skin collagen type I and type III, Biochim Biophys Acta 1804 (2010) 731-739.

The turnover of elastic fibers





Elastases

Matrix metalloproteinases



Elastases Serine proteases **Proteinase 3** Human leukocyte **Cathepsin G** elastase (HLE) (PR3) (CG) Released in Stored in Chronic obstructive response to azurophilic pulmonary disease inflammation granula of • Pulmonary emphysema processes, • Atherosclerosis human degradation of neutrophils Tumor progression pathogens

Enzymatic degradation of elastin

How do the enzymes act on elastin and tropoelastin?Cleavage site specificities

Cleavage behavior

Are there any differences between elastin isolated by different methods?

Which peptides are released during degradation of elastin?

• Bioactive motifs?

A. Heinz, M.C. Jung, L. Duca, W. Sippl, S. Taddese, C. Ihling, A. Rusciani, A.S. Weiss, R.H.H. Neubert, C.E.H. Schmelzer, Degradation of tropoelastin by matrix metalloproteinases: cleavage site specificities and release of matrikines, *FEBS Journal*, 277(8), 1939-1956, 2010.

Cleavage behavior

- 1 MAGLTAAAPR PGVLLLLLSI LHPSRPGGVP GAIPGGVPGG VFYPGAGLGA 50
- 51 LGGGALGPGG KPLKPVPGGL AGAGLGAGLG AFPAVTFPGA LVPGGVADAA 100
- 101 AAYKAAKAGA GLGGVPGVGG LGVSAGAVVP OPGAGVKPGK VPGVGLPGVY 150
- 151 PGGVLPGARF PGVGVLPGVP TGAGVKPKAP GVGGAFAGIP GVGPFGGPQP 200
- 201 **GVPLGYPIKA PKLPGGYGLP 13 YTTGKLPYGY GPGGVAGAAG KAGYPTGTGV** 250
- 251 GPQAAAAAAA KAAAKFGAGA AGVLPGVGGA GVPGVPGAIP GIGGIAGVGT 300
- 301 PAAAAAAAA AKAAKYGAAA GLVPGGPGFG PGVVGVPGAG VPGVGVPGAG 350
- 351 IPVVPGAGIP GAAVPGVVSP EAAAKAAAKA AKYGARPGVG VGGIPTYGVG 400
- 401 AGGFPGFGVG VGGIPGVAGV PSVGGVPGVG GVPGVGIŠPE AQAAAAAKAA 450
- 451 KYGVGTPAAA AAKAAAKAAQ FGLVPGVGVA PGVGVAPGVG VAPGVGLAPG 500
- 501 VGVAPGVGVA PGVGVAPGIG PGGVAAAAKS AAKVAAKAOL RAAAGLGAGI 550
- 551 PGLGVGVGVP GLGVGAGVPG LGVGAGVPGF GAVPGALAAA KAAKYGAAVP 600
- 601 GVLGGLGALG GVGIPGGVVG AGPAAAAAAA KAAAKAAOFG LVGAAGLGGL 650
- 651 GVGGLGVPGV GGLGGIPPAA AAKAAKYGAA GLGGVLGGAG OFPLGGVAAR 700
- 701 PGFGLSPIFP GGACLGKACG RKRK

MMP-7: 84 peptides

16

- ▲ MMP-9: 74 peptides
- ▲ MMP-12: 132 peptides

Sequence coverages

MMP-7: 71 % MMP-9: 60 % MMP-12: 81 % A. Heinz, M.C. Jung, L. Duca, W. Sippl, S. Taddese, C. Ihling, A. Rusciani, A.S. Weiss, R.H.H. Neubert, C.E.H. Schmelzer, Degradation of tropoelastin by matrix metalloproteinases: cleavage site specificities and release of matrikines, *FEBS Journal*, 277(8), 1939-1956, 2010.

Cleavage site preferences

 $\rm NH_2$

У ---- КООН

Amino acid	Ρ	4 /	%	Ρ	₃ /	%	Ρ	2 /	%	Ρ	1 /	%	P	i'/	%	Ρ	2' /	%	P	3' / '	%	P.	4'/	%
G		10	27	23	14	15		31	12		38	33	12	14	7			1	30		24	32	23	21
Α	18	27	27	17	30	4	27	30		15	13		3	19	26	13	29	30	29	14	12	22	36	35
V	8	9	10	13	9	6	2	2	1	3	11	7	12	9	9	13	8	7	12	3	5	17	6	11
L	5	6	6	10	13	6	7	11	6	5	3	1	48	22	20	3	9	4	5	5	5	7	14	7
L	3	2	1	3	2	1	0	0	2	3	3	1	3	3	2	0	0	1	0	0	0	3	3	3
F	2	0	1	2	0	0	8	5	4	2	0	1	3	0	4	2	8	2	2	3	3	2	0	1
Υ	5	3	3	0	2	0	2	5	4	5	3	0	5	6	9	2	6	1	3	2	4	3	3	2
K	5	3	11	2	3	1	0	2	4	2	0	8	2	11	12	5	8	11	0	2	3	2	5	7
Ρ	15	5	8	18	23	24	13	8	2	7	6	4	10	8	5	0	0	2	9	6	5	9	8	8
MMP-	7	9	12	7	9	12	7	9	12	7	9	12	7	9	12	7	9	12	7	9	12	7	9	12

Occurrence of different amino acids at the substrate positions $P_1 - P_4$ and $P_1' - P_4'$ after digestion with MMP-7, MMP-9, and MMP-12. Values are based on the number of cleavage sites identified upon mass spectrometric analysis of the digests.

A. Heinz, M.C. Jung, L. Duca, W. Sippl, S. Taddese, C. Ihling, A. Rusciani, A.S. Weiss, R.H.H. Neubert, C.E.H. Schmelzer, Degradation of tropoelastin by matrix metalloproteinases: cleavage site specificities and release of matrikines, *FEBS Journal*, 277(8), 1939-1956, 2010.

Molecular modeling

- 1 MAGLTAAAPR PGVLLLLLSI LHPSRPGGVP GAIPGGVPGG VFYPGAGLGA 50
- 51 LGGGALGPGG KPLKPVPGGL AGAGLGAGLG AFPAVTFPGA LVPGGVADAA 100
- 101 AAYKAAKAGA GLGGVPGVGG LGVSAGAVVP OPGAGVKPGK VPGVGLPGVY 150
- 151 PGGVLPGARF PGVGVLPGVP TGAGVKPKAP GVGGAFAGIP GVGPFGGPQP 200
- 201 **GVPLGYPIKA PKLPGGYGLP YTTGK LPYG YGPG GVAGAAG KAGYPTGTGV** 250
- 251 GPQAAAAAAA KAAAKFGAGA AGVLPGVGGA GVPGVPGAIP GIGGIAGVGT 300
- 301 PAAAAAAAAA AKAAKYGAAA GLVPGGPGFG PGVVGVPGAG VPGVGVPGAG 350
- 351 IPVVPGAGIP GAAVPGVVSP EAAAKAAAKA AKYGARPGVG VGGIPTYGVG 400
- 401 AGGFPGFGVG VGGIPGVAGV PSVGGVPGVG GVPGVGISPE AQAAAAAKAA 450
- 451 **KYGVGTPAAA AAKAAAKAAQ FGLVPGVGVA PGVGVAPGVG VAPGVGLAPG** 500
- 501 VGVAPGVGVA PGVGVAPGIG PGGVAAAAKS AAKVAAKAQL RAAAGLGAGI 550
- 551 PGLGVGVGVP GLGVGAGVPG LGVGAGVPGF GAVPGALAAA KAAKYGAAVP 600
- 601 GVLGGLGALG GVGIPGGVVG AGPAAAAAA KAAAKAAQFG LVGAAGLGGL 650
- 651 GVGGLGVPGV GGLGGIPPAA AAKAAKYGAA GLGGVLGGAG OFPLGGVAAR 700

701 PGFGLSPIFP GGACLGKACG RKRK

Tropoelastin (isoform 2)



Interaction of the natural substrate **LPYGYGPG** (residues 226-233 from tropoelastin isoform 2) and the MMP-12 active site. The molecular surface of the binding pocket is colored according to electrostatic potential (red: negative electrostatic potential, blue: positive electrostatic potential).

Does HLE degrade elastin?



Cleavage behavior of NSPs - tropoelastin

- 1 MAGLITAAAPR FOVLLLILEI LHFSRFGGVP GAIPGGVPGG VFYPGAGLGA 50
- 51 LGGGALGPGG KPLKFVPGGL AGAGLGAGLG AFFAVTFPGA LVFGGVADAA 100
- 101 AAYKAAKAGA GLGGVPGVGG LGVSAGAVVP OPGAGVKPGK VPGVGLPGVY 150
- 151 PGGVLPGARF PGVGVLPGVP TGAGVKPKAP GVGGAFAGIP GVGPFGGPQP 20
- 201 GVPLGYPIKA PKLPGGYGLP YTTGKLPYGY GPGGVAGAAG KAGYPTGTGV 250
- 251 GPOAAAAAAA KAAAKFGAGA AGVLPGVGGA GVPGVPGAIP GIGGIAGVGT 300
- 301 PAAAAAAAAA AKAAKYGAAA GLYPGGPGFG PGYYGYPGAG YPGYGYPGAG 350
- 351 IPVVPGAGIF GAAVPGVVSP EAAAKAAAKA AKYGARPGVG VGGIPTYGVG 400
- 401 AGGFPGFGVG VGGIPGVAGV PSVGGVPGVG GVPGVGISPE AQAAAAAKAA 450
- 451 KYGVGTPAAA AAKAAAKAAO FGLVPGVGVA PGVGVAPGVG VAPGVGLAPG 500
- 501 VGVAPGVGVA PGVGVAPGIG PGGVAAAAKS AAKVAAKAOL RAAAGLGAGI 550
- 551 PGLGVGVGVP GLGVGAGVPG LGVGAGVPGF GAVPGALAAA KAAKYGAAVP 600
- E01 GVLGGLGALG GVGIPGGVVG AGPAAAAAAA KAAAKAAQFG LVGAAGLGGL 650
- 651 GVGGLGVPGV GGLGGIPPAA AAKAAKYGAA GLGGVLGGAG OFPLGGVAAR 700
- 701 PGFGLSPIFF GGACLGRACG RKRK HIE PR3 Tropoelastin isoform 2

CG

HLE: 408 peptides
 PR3: 305 peptides
 CG: 197 peptides

Sequence coverages HLE: 94 % PR3: 99 % CG: 96 %

22

Digestion with HLE



Characterization of elastin from different tissues



Challenges

... with elastin peptides

- (1) Peptides mainly hydrophobic
- (2) Proteolysis time consuming, not digestable with specific proteases
- (3) Many of the peptides have equal or almost equal masses

25



Search for $M_r = 711.3915$

Ma	155	sea	rch	res	ult	s																								- 15	
MAG	LT	A A	A P	R P	GU	JLI	L	LL	SI	LH	ΡS	RP	° G	GV	P G	A I	PG	GV	PG	GV	FΥ	PGI	A G L	G F	ILG	G G 711,	AL 39	GP	G G 60 711,39	6	pin
KPLI	KP	U P	G G	LA	Gf	G	L G 7	A G	LG	AF	P A	UT	F	P G	AL	V P	G G	VA	DA	AA	ΑY	KAI	A K A	I G F	GL	GG	VP	GV	G G 12	0. 1,39	-
L G U : 711(3	5 A 139	GA	υU	PQ	PO	G A I	GV	K P	G K	VP	GV	GL	. P	GV	ΥP	G G	VL	PG	A R	FP	GV	GVI	LPG	VF	т	A G	UK	PK	AP 18	9 0	
GVG	GA	FA	GI	P G	V	3 P I	FG	G P	Q P	GV	PL	G Y	P	I K	A P	KL	P G	GY	GL	PΥ	ΤT	GKI	LPY	GY	' G F	GG	VA	G A I	A G 24	0	
KAG	ΥP	T G	T G	VG	P () A i	AA	A A	AA	KA	A A	KF	G	A G	A A	GV	LP	G V 710	G G 3599	A G	VP	GV	P G A 711,39	ĪF	• G 1	GG	IA	GV	G T 30	0	
PAA	A A	A A	A A	A A	K f	A	(Y	GA	AA	GL	U P	GG	F P	G F 1339	G P	G V	VG	UP	G A	G V	P G 11,39	VG	711	A (I F	UU	PG	A G	I P 36	:0	
GAA	U P 7	G U 1,39	U S	ΡE	Af	A I	(A	A A	KA	A K	Y G	AR	8 P	GV	GV	G G	I P	ΤY	GV	G A	G G	FPI	GFG	UC	. U G	GI	PG	U A I 711,3	GU 42 9	0	
°SŲ	GG	U P	GV	G G	VF	G	JG	15	PE	AQ	AA	AA	A	KA	A K	¥ G	AA	GA	GV	LG	GL	VPI	GPQ	AA	VF	GV	PG	TG	G V 48	0	
GV	GT	PA	A A	A A	Kf	A A	A K	A A	QF	GL	U P	GU	JG	VA	PG	V G	U A 711	P G 39	VG	V A 711,	P G 39	VG	A P 711,39	GL	GL	A P	GV	G U I	AP 54 11,39	0	
GVG	U A 711,3	P G 9	IG	P G	GU	A	A A	A K 711	S A 39	A K	VA	AK	(A	QL	RA	AA	GL	GA	GI	PG	L G 711,3	U G I	VGV	PE	: L 0	V G	A G	V P I	GL 60	0	
GVG	A G	U P	GF	G A	Gf	D	EG	U R	RS	LS	PE	LR	8 E	G D	P S	55	QH	LP	S T	PS	S P	RVI	PGA	ILF	AA	KA	AK	YG	A A 66	0	
V P G	VL	G G	LG	AL	G(30	G I	P G	GV	V G 711,3	A G	PA	A	A A 7	A A 11,39	A K	AA	A K	AA	QF	GL	VGI	A A G	:L0	61	G V	GG	LG	UP 72	Ö	
G U G 711,3	G L 7147	G G 9,39	I P	P A	Af	A	(A	A K	Y G	AA	GL	GC	: U	LG	GA	G Q	FP	LG	GV	AA	RP	GFI	GLS	: P 1	FF	G G	A C	LGI	K A 78	0	
CGRI	KR	7 K 78	1,39 6	55																		•	Tr	0	р	00	ela	as	sti	n	
Anal	yze	R	epo	rt	1																				-						-

-	Mass	search result	5						
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V	Ħ	Mass	Ch.	Dev.	From-	To	Se	quence	
	Sear	ching for	mass:	71	1,3915	+/-0.	, 00	07	
	0	711,3915	[1]	0	573-	581	AA	AGLGAGIPG	LG
	0	711,3915	[1]	0	548-	556	PG	IGPGGVAAA	AK
	0	711,3915	[1]	0	535-	543	AP	GVGVAPGVG	VA
	0	711,3915	[1]	O	529-	537	AP	GVGVAPGVG	VA
	0	711,3915	[1]	O	517-	525	AP	GVGVAPGVG	LA
	0	711,3915	[1]	O	511-	519	AP	GVGVAPGVG	VA
	0	711,3915	[1]	O	505-	513	VP	GVGVAPGVG	VA
	0	711,3915	[1]	O	672-	680	AL	GGVGIPGGV	VG
	0	711,3915	[1]	O	680-	688	GG	VVGAGPAAA	AA
	0	711,3915	[1]	O	408-	416	GF	GVGVGGIPG	VA
	0	711,3915	[1]	O	715-	723	GV	GGLGVPGVG	GL
	0	711,3915	[1]	O	713-	721	GL	GVGGLGVPG	VG
	0	711,3915	[1]	O	719-	727	LG	VPGVGGLGG	IP
	0	711,3915	[1]	0	718-	72.6	GL	GVPGVGGLG	GI
	0	711,3915	[1]	0	716-	724	VG	GLGVPGVGG	LG
	0	711,3915	[1]	O	277-	285	PG	VGGAGVPGV	PG
	0	711,3915	[1]	0	2.68-	276	FG	AGAAGVLPG	VG
	0	711,3915	[1]	0	356-	364	VP	GAGIPGAAV	PG
	0	711,3915	[1]	0	338-	346	VP	GAGVPGVGV	PG
	0	711,3915	[1]	0	267-	275	KF	GAGAAGVLP	GV
	0	711,3915	[1]	0	332-	340	GP	GVVGVPGAG	VP
	0	711,3915	[1]	0	318-	32.6	YG	AAAGLVPGG	PG
	0	711,3915	[1]	0	317-	325	KY	GAAAGLVPG	GP
	0	711,3915	[1]	0	114-	122	LG	GVPGVGGLG	VS
	0	711,3915	[1]	0	113-	121	GL	GGVPGVGGL	GV
	0	711,3915	[1]	O	112-	120	AG	LGGVPGVGG	LG
	0	711,3915	[1]	O	111-	119	GA	GLGGVPGVG	GL
	0	711,3915	[1]	0	67-	75	PV	PGGLAGAGL	GA
	0	711,3915	[1]	0	50-	58	LG	ALGGGALGP	GG
	0	711,3915	[1]	0	44-	52	FY	PGAGLGALG	GG
	deces.	andre stande strekter	771871						
	Vo m	odificatio	on list	t enak	led				
A	nalyze	Report			orna Miccarle				



Challenges

... with elastin peptides

- (4) Many repetitive regions
 - ⇒ High similarity between peptides and thus between fragmentation patterns

..GL**GVPGVGG**.. 711.39 Da

26

..LG**GVPGVGG**.. 711.39 Da

..GGVPGVGGL.. 711.39 Da

(5) Cross-links and other PTMs

Initial situation



x 150 samples x 7 = 1050

- ➤ Skin
- Aorta
- ➤ Cartilage
- Intervertebral disc
- ➤ WBS skin
- ➤ WBS aorta













IonHunter



Supervised and unsupervised biomarker identification



$[M+H]^+$	Sequence	Start/Stop residue (IF 9)	Pro residue (IF 9)	Ratio
814.45 Da	F. AGIPGVGPF .G	187 - 195	190	AUC _{830.44} /AUC _{814.45}
830.44 Da	F. AGIpGVGPF .G	187 - 195	190	= HyP ₁₉₀ /P ₁₉₀
939.53 Da	L. GGVGIPGGVVGA .G	678 - 689	683	AUC _{955.52} /AUC _{939.53}
955.52 Da	L. GGVGIpGGVVGA .G	678 - 689	683	$= HyP_{683}/P_{683}$

Identified marker peptides

About 20 identified marker peptides that give insight into specific features of elastin such as

- > Age and/or degeneration status
- Type of tissue
- Existence and level of PTMs
- Presence of absence of certain domains
- Status of known point mutations

Characterization of crosslinking pattern of elastin

Recovered peptides from degradation with elastases

Cross-linking in elastin

Brown-Augsburger, P., Tisdale, C., Broekelmann, T., Sloan, C. and Mecham, R. P. (1995) J Biol Chem 270 (30): 17778-17783.

Cross-linking of three tropoelastin molecules at domains 10, 19 and 25

Woods (1981).

Cross-linking in elastin

41

Transfer-Coll. 50 eV, Mobility TOF, MS/MS 884.55

42

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

Scores for different cross-linked species containing dehydrolysinonorleucine. The score obtained for the correct peptide sequence is compared with scores obtained for its permutated decoy sequences.

4<u>3</u>

Minimized structure of a desmosinecontaining peptide after 20 ns of molecular dynamics simulations using the AMBER program. The central desmosine ring system is shown in white, the backbone of the peptide is shown as pink ribbon and hydrogen bonds are shown as green line.

Cross-linked tropoelastin

A. P. McGrath, S. M. Mithieux, C. A. Collyer, J. G. Bakhuis, M. van den Berg, A. Sein, A. Heinz, C. Schmelzer, A. S. Weiss, and J. M. Guss, Biochemistry 50 (2011) 5718-5730.

MALDI TOF/TOF tandem mass spectrum of propionylated demosine ([M+H]+ 750.39) detected in ANAO cross-linked tropoelastin.

Summary

- Isolation of intact and pure elastic fibers from single biopsies allows for elastolytic studies *in vitro*
- Intact elastin from young individuals (< 40 years) cannot be degraded by human leukocyte elastase
- However, elastin from older patients is susceptible to HLE

With increasing age, elastic fibers lose their resistance against proteolytic degradation \rightarrow increased turnover

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 Clear differences in the cleavage pattern of elastin from patients of different ages

Molecular clocks

 Increased hydroxyproline levels for IVD elastin and decreased levels for elastin from WBS patients

Summary

 Development of new algorithms for biomarker discovery taking into account common limitations and features of LC-MS measurements such as non-linear retention time shifts

- In vitro formation of dehydrolysinonorleucine and desmosine containing peptides
 - Scoring designed for the specific demands of the fragmentation pattern of cross-linked elastin peptides
 - Insights into the cross-linking of tropoelastin monomers and elastin-degrading processes
 - Development of directed therapies against elastin-degrading diseases
 - Biomaterials (stents, soft tissue repair matrix)

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