Elastolytic mechanism of a novel M23 metalloprotease pseudoalterin from deep-sea *Pseudoalteromonas* sp. CF6-2: cleaving not only the glycyl bonds in the hydrophobic regions, but also the peptide bonds in the hydrophilic regions involved in crosslinking

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Running title: Elastolytic mechanism of a novel M23 metalloprotease

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Supplementary Data

Procedure	Volume	Protein	Total protein	Total Activity	Specific	Purification	Yield
	(ml)	concentration	(mg)	(U)	Activity	(fold)	(%)
		(mg/ml)			(U/mg)		
Culture	100	0.1123	11.23	1504	133.93	1	100
supernatant							
DEAE	20	0.126	2.52	506.6	201.03	1.50	33.68
sepharose							
fast flow							

Table S1. Purification of the protease secreted by Pseudoalteromonas sp. CF6-2



Fig. S1. Purification of pseudoalterin by ion-exchange chromatography on a DEAE-Sepharose Fast Flow column. •, protein concentration; \circ , relative protease activity; –, NaCl concentration.



Fig. S2. Analysis of the purity of pseudoalterin by SDS-PAGE.1, proteins in *Ps.* sp. CF6-2 culture supernatant; 2, pseudoalterin purified by ion exchange chromatography; M: marker.





Fig. S3. Effect of temperature and pH on the activity of pseudoalterin. A, Effect of pH on pseudoalterin activity. The optimum pH was determined by measuring the activities of pseudoalterin with elastinorcein at 30°C in Na₂HPO₄-NaH₂PO₄ buffer at pHs ranging from 6.5 to 8, barbital sodium-HCl buffer at pHs ranging from 7.5 to 9.6 and NaHCO₃-NaOH buffer ranging from 9.6 to 11.0. B, Effect of temperature on pseudoalterin activity. C, Effect of temperature on pseudoalterin activity in artificial sea water. The optimum temperature was determined by measuring the activities of pseudoalterin with elastinorcein in 50 mM Tris buffer (pH 9.0) or in artificial sea water at temperatures ranging from 0 to 45°C. The data shown are the mean of three repeats with standard deviations $\leq 5\%$.



Fig. S4. Effect of temperature on the thermostability of pseudoalterin. The concentration of the incubated pseudoalterin is 0.1 mg/mL. The data shown are the means of three repeats with standard deviations $\leq 5\%$.

ATGGGGTTTTATAAAAGGGAACAAAAAG -1

M	N	K	Н	L	L	Т	L	A	v	T	T	G	L	G	F	s	s	I	A
TTT	GCC	GGT	GTG	CAT	AAC	CAT	GAA	ACG	TTT	GAA	TTT	TCA	GAC	CAA	GCA	GTA	GAA	CAA	СТ
F	A	G	v	н	N	н	E	т	F	E	F	s	D	Q	A	v	E	Q	L
		t —												-					
AAT	CTT	AAT	TCA	TTA	TTA .	ATT	ATG	GAT	GAC	CAA	ACA	TTT	GTA	TTT .	AAT	AAT	GAC	СТА	TTA
N	L	N	S	L	L	I	М	D	D	Q	т	F	v	F	N	N	D	L	L
AAT	GAA	GAC	TGG	GAT	AAC	TAC	TTC	GCG	TCG	TAT	GCC	CCT	GAA	CTT	CAA	AGT	AAA	CAA	GC
N	Е	D	W	D	N	Y	F	А	s	Y	А	Р	E	L	Q	S	K	Q	А
TTT	ATA	СТА	CAC	TGG	GCT	GGC	TAT	TAT	AGT	ATT	AAC	CCA	AAA	GTG	ATT	TTG	GCA	CTG	ATT
F	I	L	н	w	Α	G	Y	Y	S	I	Ν	Р	к	v	Ι	L	Α	L	I
GAA	CAG	G CAA	AGT	GAC	GGA	CTO	TCT	GAT	CCT	TCG	GTT	GAA	TTA	GAA	AGO	GTC	TTT	AAA	AA
E	Q	Q	S	Е	G	L	S	D	Р	S	V	Е	L	Е	S	V	F	К	N
ATC	TCT	GAC	AAG	CAA	GGA	TTT	GAG	GAG	CAG	GTT	AA/	GAG	C GTI	GTA	TTT	AAA	TTA	AGT	CA
I	S	D	к	Q	G	F	E	E	Q	V	к	D	V	v	F	К	L	S	Q
CGT	TTC	TAT	GCC	TTT	AAA (CAC	TGG	CAA	GAG	CAA	GCC	GTA	AAG	CAT	GAT	AAA	AAC	TCA	AA
R	F	Y	А	F	к	н	W	Q	Е	Q	Α	v	К	Н	D	к	N	S	N
TCC	ATA	AAG	CAT	TTA	ATT	AGG	CCT	TCA	CAA	GTG	AGT	ACT	GCC	GCT	ACA	GCT	GCG	CTT	GCC
S	I	K	н	L	I	R	Р	S	Q	V	S	Т	Α	Α	Т	Α	Α	L	Α
AGC	ATG	ATG	AGT	AAG	CAG	CAC	AA7	r ctg	CAT	GGT	CAC	GC/	A AA	GA	г тст	Γ ΤΤΑ	ACT	CGC	TT
S	M	M	S	K	Q	Н	N	L	Н	G	Q	A	N	D	S	L	Т	R	F
TTA	GAT	ATA	TTC	GAG	CAA	CTA	TCA	CCC	GAA	CAG	TCT	CTT	ATC	TTG	AAC	ACA	GAC	CAA	GTC
L	D	I	F	E	Q	L	S	Р	E	Q	S	L	I	L	N	Т	D	Q	V
ACT	TTC	TCA	GGG	GAA	GAG	CAG	TCG	GTT	CAA	GCC	ACA	TTT	ACT	ATG	AAC	TTA	CCT	TGG	TCT
Т	F	S	G	E	E	Q	S	V	Q	A A	Т	F	Т	М	N	L	Р	w	S
					-		- Pro	o-seque	ence	M	ature	Enzym	ne	•					
CAA	GGG) TAT	TAT	TGG	TAT	AGT	GGT	GGA	GCG	CAT	TCA	AAC	ACT	GGC	TCA	GG1	TAT	CCT	TAT
Q	G	Y	Y	W	Y	s	G	G	Α	н	S	Ν	Т	G	S	G	Y	Р	Y
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тст	TCA	тта	GAC	TTT	AAC A	AAT	GGT	тст с	GGT	GGA	TGG	GGA	AGC	AAT	ACG	CCT	TGG	GTT	CAA
TCT	TCA S	TTA L	GAC D ▲	TTT . F	AAC A	AAT N	GGT G	TCT O	GGT G	GGA G	төө W	GGA G	AGC S	AAT N	ACG T	Р	тбб W	GTT V	CAA Q
TCT S	TCA S	TTA L Zir	GAC D nc Lig	F	N	AAT N	GGT G	S	GGT G	GGA G	TGG W	GGA G	AGC S	AAT	ACG T	Р	TGG W	GTT V	CAA Q
TCT S GCC	TCA S GCA	TTA L Zii CAT	GAC D A ne Lig GGT	F and GGG	AAC A	AAT N ATC	GGT G ACT	CGT	GGT G	GGA G TCG	TGG W TCA	GGA G TGT	AGC S AAT	AAT N ATA	ACG T CGG	CCT P GTA	TGG W ACG	GTT V CAC	CAA Q TCA
S GCC	TCA S GCA A	TTA L Zit CAT H	GAC D ne Lig GGT G	F F and GGG G	AAC A N GTC V	AAT N ATC I	GGT G ACT T	CGT R	GGT G TTT F	GGA G TCG S	TGG W TCA S	GGA G TGT C	AGC S AAT N	AAT N ATA I	ACG T CGG R	CCT P GTA V	TGG W ACG T	GTT V CAC H	CAA Q TCA S
TCT S GCC A	TCA S GCA A	TTA L Zii CAT H	GAC D Ane Liga GGT G	TTT F and GGG G	AAC A N GTC V	AAT N ATC I	GGT G ACT T	CAC	GGT G G TTT F	GGA G TCG S	TCA S	GGA G TGT C	AGC S AAT N	AAT N ATA I	ACG T CGG R	GTA V	TGG W ACG T	GTT V CAC H	CAA Q TCA S
TCT S GCC A AGT	TCA S GCA A GGC	TTA L Zii CAT H TTT	GAC D A ne Lig GGT G GCA	TTT F and GGG G ACT T	AAC A N GTC V AAT	AAT N ATC I TAT	GGT G ACT T TAC	CGT R CAC	GGT G G TTT F ATG	GGA G TCG S TCT	TGG W TCA S AAC	GGA G TGT C TTA	AGC S AAT N CAA	AAT N ATA I TAC	ACG T CGG R AAT	GTA V AAT	TGG W ACG T GGT	GTT V CAC H GAC	CAA Q TCA S ACT
TCT S GCC A AGT S	TCA S GCA A GGC G	TTA L Zii CAT H TTT F	GAC D Anc Lig GGT G GCA A	F F and GGG G ACT T	AAC / N GTC V AAT N	AAT N ATC I TAT Y	GGT G ACT T TAC Y	CGT R CAC	GGT G G TTT F ATG M	GGA G TCG S TCT S	TGG W TCA S AAC N	GGA G TGT C TTA L	AGC S AAT N CAA Q	AAT N ATA I TAC Y	ACG T CGG R AAT N	GTA V AAT N	TGG W ACG T GGT G	GTT V CAC H GAC D	CAA Q TCA S ACT
TCT S GCC A AGT S	TCA S GCA A GGC G	TTA L Ziu CAT H TTT F	GAC D Anc Liga GGT G GCA A	F F and GGG G ACT T	AAC / N GTC V AAT N	AAT N ATC I TAT Y	GGT G ACT T TAC Y	CGT R CAC H	GGT G G TTT F ATG M	GGA G TCG S TCT S	TGG W TCA S AAC N	GGA G TGT C TTA L	AGC S AAT N CAA Q	AAT N ATA I TAC Y	ACG T CGG R AAT N	GTA V AAT N	TGG W ACG T GGT G	GTT V CAC H GAC D	CAA Q TCA S ACT T
TCT S GCC A AGT S GTG	TCA S GCA A GGC G CAG	TTA L Zia CAT H TTT F CCA	GAC D Ance Liga GGT G GCA A GGA	TTT . F and GGG G ACT T ACG	AAC J N GTC V AAT N TTG	AAT N ATC I TAT Y CTA	GGT G ACT T TAC Y GGT	CGT R CAC H CGT	GGT G G TTT F ATG M TAT	GGA G TCG S TCT S GCG	TCA S AAC N AAT	GGA G TGT C TTA L AGT	AGC S AAT N CAA Q TAT	AAT N ATA I TAC Y AAC	ACG T CGG R AAT N CAA	GTA V AAT N GCA	ACG T GGT G CTG	GTT V CAC H GAC D TGC	CAA Q TCA S ACT T C GA
TCT S GCC A AGT S GTG V	TCA S GCA A GGC G CAG Q	TTA L Zii CAT H TTT F CCA P	GAC D Ane Lig GGT G GCA A GGA GGA	F and GGG G ACT T ACG T	AAC A N GTC V AAT N TTG L	AAT N ATC I TAT Y CTA L	GGT G ACT T TAC Y GGT G	CGT R CAC H CAC R	GGT G G TTT F ATG M TAT Y	GGA G TCG S TCT S GCG A	TGG W TCA S AAC N AAT N	GGA G TGT C TTA L S	AGC S AAT N CAA Q TAT Y	AAT N ATA I TAC Y AAC N	ACG T CGG R AAT N CAA Q	GTA V AAT N AGCA A	TGG W ACG T GGT G CTG L	GTT V CAC H GAC D TGC C	CAA Q TCA S ACT T C GA E
TCT S GCC A AGT S GTG V	TCA S GCA A GGC G CAG Q	TTA L Ziu CAT H TTT F CCA P	GAC D nc Lige GGT G GCA A GGA G GGA	F and GGG G ACT T ACG T	AAC / N GTC V AAT N TTG L	AAT N ATC I TAT Y CTA L	GGT G ACT T TAC Y GGT G	CGT CGT R CAC H CGT R	GGT G TTT F ATG M TAT Y	GGA G TCG S TCT S GCG A	TCA S AAC N AAT N	GGA G TGT C TTA L AGT S	AGC S AAT N CAA Q TAT Y	AAT N ATA I TAC Y AAC N	ACG T CGG R AAT N CAA Q	GTA V AAT N GCA A	ACG T GGT G CTG L	GTT V CAC H GAC D GAC C	CAA Q TCA S ACT T C GA
TCT S GCC A AGT S GTG V GGC	TCA S GCA A GGC G CAG Q GGA	TTA L Zii CAT H TTT F CCA P CAA	GAC D D GGT GGT GCA A GGA GGA G GGA G C A	F F and GGG G ACT T ACG T T TCT s	AAC / N GTC V AAT N TTG L GGT	AAT N ATC I TAT Y CTA L CCA	GGT G ACT T TAC Y GGT G CAC	CGT R CAC H CGT R CGT R	GGT G TTT F ATG M TAT Y CAC	GGA G TCG S TCT S GCG A TTC	TCA S AAC N AAT N ACT	GGA G TGT C TTA L AGT S TTA	AGC S AAT N CAA Q TAT Y TTA	AAT N ATA I TAC Y AAC N CAA	ACG T CGG R AAT Q AAT	GTA V AAT N GCA A GGT	ACG T GGT G CTG L CAG	GTT V CAC H GAC D TGC C CAA	CAA Q TCA S ACT T C GA E GT
TCT S GCC A AGT S GTG V GGC G	TCA S GCA A G G CAG Q G G G G G G G G G	TTA L Zii CAT H TTT F CCA P CAA Q	GAC D A ne Lige GGT GCA A GGA G A GGA G A C S	F and GGG G ACT T ACG T T TCT S	AAC / N GTC V AAT N TTG L GGT G	AAT N ATC I TAT Y CTA L CCA P	GGT G ACT T TAC Y GGT G CAC	CAC H CAC CAC CAC CAC CAC	GGT G TTT F ATG M TAT Y CAC H	GGA G TCG S TCT S GCG A TTC F	TGG W TCA S AAC N AAT N ACT T	GGA G TGT C TTA L TTA L TTA L	AGC S AAT N CAA Q TAT Y TTA L	AAT N ATA I TAC Y AAC N CAA Q	ACG T CGG R AAT N CAA Q AAT N	GTA V AAT N GCA A GGT G	ACG T GGT G CTG L CAG Q	GTT V CAC H GAC D TGC C C C AA Q	CAA Q TCA S ACT T C GA E GT V
TCT S GCC A AGT S GTG V GGC G	TCA S GCA A GGC G CAG Q CAG Q CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG	TTA L Zii CAT H TTT F CCA P CAA Q	GAC D A ne Lige GGT G GCA A GGA G A C S	F and GGG G ACT T ACG T TCT S	AAC / N GTC V AAT N TTG L GGT G Act	AAT N ATC I TAT Y CTA L CCA P tive Si	GGT G ACT T TAC Y GGT G CAC H tet	CGT R CAC H CAC R CAC R CAC R CAC R CAC	GGT G G TTT F ATG M TAT Y CAC H CAC	GGA G TCG S TCT S GCG A TTC F gand	TGG W TCA S AAC N AAT T	GGA G TGT C TTA L AGT S TTA L	AGC S AAT N CAA Q TAT Y TTA L	AAT N ATA I TAC Y AAC N CAA Q	ACG T CGG R AAT N CAA Q AAT	GTA V AAT N GCA A GGT G	ACG T GGT G CTG L CAG Q	GTT V CAC H GAC D TGC C C C AA Q	CAAA Q TCAA S ACT T T CAAC CAAC CAAC
TCT S GCC A AGT S GTG V GGC G TCA	TCA S GCA A GGC G CAG Q CAG Q CAG CAG CAG CAG CAG CAG CAG CAG CAG CAG	TTA L Zin CAT F CCA P CAA Q CAT	GAC D A ne Lig GGT GCA A GGA G A C S AAC	TTT F and GGG G ACT T ACG T TCT S CGT	AAC / N GTC V AAT N TTG L GGT G Act TAT	AAT N ATC I TAT Y CTA L CCA P tive Si ATC	GGT G ACT T TAC Y GGT G CAC H te A GT	CAC R CAC H CAC R CAC R CAC R CAC R CAC R CAC R CAC R CAC R	GGT G G TTT F ATG M TAT Y CAC H CAC	GGA G TCG S TCT S GCG A TTC F gand CGT P	TCA S AAC N AAT T ATT	GGA G TGT C TTA L AGT S TTA L GAT	AGC S AAT N CAA Q TAT Y TTA L GTT	AAT N ATA I TAC Y AAC N CAA Q GGT	ACG T CGG R AAT N AAT N AAT	GTA V AAT N GGC/ A GGT G AGT	ACG T GGT G CTG L CAG Q AAC	GTT V CAC H GAC D TGC C C C AA Q TAT	CAA Q TCA S ACT T C GA E GT V V GAT
TCT S GCC A AGT S GTG V GGC G TCA S	TCA S GCA A G G CAG Q CAG G G TTA L	TTA L Zii CAT H : TTTT F : CCA P CAT H	GAC D A nec Ligg GGT G GCA A G GCA A G GCA A C C S A A C S A A C S	FTTF , F and GGG G ACT T ACG T S CGT R	AAC J N GTC V AAAT N TTG L GGT G Act TAT Y	AAT N ATC I TAT Y CTA L CCA P tive Si ATC I	GGT G ACT T TAC Y GGT G CACC H te★ AGT S	CGT R CAC H CAC H CGT R GTA V Z AAT N	GGT G G TTTT F ATG M TAT Y CACC H time Lic TAC Y	GGA G TCG S TCT S GCG A CGT F gand CGT R	TCA S AAC N AAT T ATT I	GGA G TGT C TTA L S TTA L GAT D	AGC S AAT N CAA Q TTA TTA L GTT V	AAT N ATA I TAC Y AAC N CAA Q GGT G	ACG T CGG R AAT N CAA Q AAT N AAT N	GTA V AAAT N GGC/ A GGT G AGT S	TGG W ACG T GGT G CAG Q AACC N	GTT V CAC H GAC D GAC C C C C C C AA Q TAT Y	CAA Q TCA S ACT T C GA C GAT D
TCT S GCC A AGT S GTG V GGC G TCA S TCA	TCA S GCA A GGC G CAG G CAG G CAG G TTA L	TTA L Zia CAT H TTTT F CCAA Q CAT H TCT	GAC D A GGT GGT G GCA A G GCA A G GCA A A C C S A A C N	F F and GGG G ACT T ACG T S CGT R	AAAC / N GTC V AAAT N TTG G G G G G G C C C C C C C C C C C C	AAT N ATC I TAT Y CTA L CCA P tive Si ATC I	GGT G ACT T TAC Y GGT G CAC H te ★ AGT	CGT R CGT R CAC H CAC R CAC R CAC T R CGT R CGT R CGT R CGT R CGT R CGT CGT CGT R CGT CGT CGT CGT R CGT CGT CGT CGT R CGT CGT CGT CGT R CGT CGT CGT CGT CGT CGT CGT CGT CGT CGT	GGT G G TTTT F ATG M TAT Y CAC H Line Li TAC Y	GGA G TCG S TCT S GCG A : TTC F gand CGT R	TCA S AAAC AAAT N ACT T AATT I	GGA G TGT C TTA L AGT S TTA L GAT D	AGC S AAT N CAA Q CAA Q TTAT Y TTA L GTT - V	AAT N ATA I TAC Y AACC N CAA Q GGT G	ACG T CGG R AAT N CAA Q AAT N AAT N	GTA V AAAT N AGC/ A GGT G G AGT S	TGG W ACG T GGT G CTG L CAG Q AACC	GTT V CAC GAC GAC C GAC C C C C C AA Q TAT Y	CAA Q TCA S ACT T C GAT C Q C C C C C C C C C C C C C C C C C
TCT S GCC A AGT S GTG V GGC G TCA S TCA	TCA S GCA A G G CAG G CAG G TTA L AAT	TTA L Zia CAT H TTTT F CAT A CAA CAA CAA CAA CAA CAA	GAC D mc Ligg GGT G GCA G GCA G GGA G GGA G GCA S A A C N	TTT , F and GGG G ACT T ACG T T TCT S CGT R AACC	AAAC / N GTC V AAAT N TTG G G G G G G G TAT - Y TTTT -	AAT N ATC I TAT Y CTA L CCA P tive Si ATC I TAT	GGT G ACT T TAC Y GGT G CAC H te TTT G TTT F	TCT C S CGT R CAC H CAC H CAC R CAC R CAC A T R CAC T R CAC T R CAC T R CAC T R CAC T R CAC T R CAT CGT R CAT C GT R CAT C GT R CAT C GT R CAT C GT R CAT C GT R CAT C GT R CAT C GT R CAT C C CAT C C CAT C C C C	GGT G G TTTT F ATG M TATT Y CACC H CACC H Zine Li CACC Y	GGA G TCG S TCT S GCG A CGT F gand CGT R AAT	TCA S AAC N AAT T AATT I GGA	GGA G TGT C TTA L AGT S TTA L GAT D CGT	AGC S AAT N CAA Q CAA Q TTA TTA L GTT V V AGA	AAT N ATA I TAC Y AACC N CAA Q GGT G G ACC	ACG T CGG R AAT N AAT N AAT N TGT	GTA V AAAT N AGC/ A GGT G GCT A	TGG W ACG T GGT G CAG CAG Q AACC N TGG	GTT V CACCH GACC C GACC C CAA Q TAT Y CGA	CAAA Q TCAS ACT T CAA E GAT C CCCC

Fig.S5. The nucleotide and deduced amino acid sequences of pseudoalterin.



B

Fig.S6. SDS-PAGE analysis of the hydrolytic activities of pseudoalterin on fibrin and gamma-globulin. A, the hydrolysis of fibrin; 1, marker; 2, 3, 4, 5, 6, 7, 8 are samples taken at 0, 20, 40, 60, 80, 100, 120 min; B, the hydrolysis of gamma-globulin; 1, marker; 2, 3, 4, 5, 6, 7, 8, 9, 10 are samples taken at 0, 10, 20, 30, 40, 50, 60, 70, 80 min.





Fig. S7. Partial LC-MS analysis of the molecular weights of the peptides released from the oxidized insulin B chain by pseudoalterin.

Peak	m/z	MH^+	Peptide ^a	RT (min)	Position
number	Measured (Da)	Matched			
		(Da)			
1	768.65	768.43	(G)SHLVEAL(Y)	3.59	9-15
2	601.64	1201.60	(C) GERGFFYTPK (A)	4.15	20-29
3	543.87	1086.57	(E) RGFFYTPKA	4.17	22-30
4	636.82	1272.64	(C)GERGFFYTPKA	4.72	20-30
5	564.92	1128.84	(E)ALYLV <u>C</u> GERG (F)	4.88	14-23
6	726.41	1451.82	$(L)V\underline{C}GERGFFYTPK(A)$	5.22	18-29
7	546.36	1091.72	(L)YLV <u>C</u> GERGF(F)	5.26	16-24
8	761.96	1522.92	(L)V <u>C</u> GERGFFYTPKA	5.30	18-30
9	783.00	1565.00	$(Y)LV\underline{C}GERGFFYTPK(A)$	5.63	17-29
10	818.48	1635.96	(Y)LV <u>C</u> GERGFFYTPKA	5.66	17-30
11	647.89	1294.78	(G)SHLVEALYLV <u>C</u> (G)	6.52	9-19
12	992.06	1983.12	(E)ALYLV <u>C</u> GERGFFYTPKA	6.67	14-30
13	921.09	1841.18	$(G) SHLVEALYLV\underline{C}GERGF(F)$	6.69	9-24
14	1274.67	2548.34	(G)SHLVEALYLV <u>C</u> GERGFFYTPKA	6.77	9-30
15	826.49	2477.47	$(G) \textbf{SHLVEALYLV} \underline{C} \textbf{GERGFFYTPK}(A)$	6.78	9-29
16	1166.00	3495.00	FVDQHLCGSHLVEALYLVCGERGFFYTPKA	8.39	1-30

Table S2. The determined molecular masses and sequences of the 16 peptides released from oxidized insulin B chain by pseudoalterin.

a The sequence of each peptide in black was determined by liquid chromatography-mass spectrometry and MASCOT MS/MS Ion Research tools. The left bracketed residue of each peptide indicates the P1-site residue of the left cleavage site. The right bracketed residue of each peptide indicates the P1'-site residue of the right cleavage site. The underline cysteine residues indicate that they are oxidized cysteine residues.



Part III. Analysis of the cleavage sites of pseudoalterin on bovine elastin fibers

Fig. S8. Partial LC-MS analysis of the molecular weights of the peptides released from bovine elastin fibers by pseudoalterin. For each peptide, the first spectra image is MS analysis and the second spectra image is the MS peak analyzed by secondary MS.

Peak	m/z	MH^+	Peptide ^a	RT (min)	Position
number	Measured	Matched		()	
	(Da)	(Da)			
1	741.67	1481.811	(G)AAGLGGVLGAGQPFPIG(G)	11.60	697-713
2	770.22	1538.833	(G)AAGLGGVLGAGQPFPIGG(G)	9.68	697-714
3	770.09	1538.854	(G)GLGVSTGAVVPQLGAGVG(A)	8.40	125-142
4	933.68	1865.991	(G)GVPGAVPGGVPGGVFFPGAGLG(G)	11.22	28-49
5	1005.93	2010.175	(G)VAPGVGVVPGVGVVPGVGVAPGIG(L)	9.98	505-528
6	556.99	1112.610	(G)GVLGAGQPFPIG(G)	9.68	696-707
7	734.65	1467.817	(G) GVGGLGVSTGAVVPQLG(A)	8.46	122-138
8	782.69	1563.886	(G)GVGGLGVSTGAVVPQLGAGVG(A)	11.47	122-142
9	1331.44	2660.550	(G)VAPGVGVVPGVGVVPGVGVAPGIGLGPGGVIG(A)	11.67	505-536
10	642.07	1282.715	(G)LGGVLGAGQPFPIG(G)	9.19	694-707
11	1011.75	2021.139	(G) LGVGGIGGVGGLGVSTGAVVPQLG(A)	11.51	115-138
12	848.19	1694.944	(G)GIGGVGGLGVSTGAVVPQLG(A)	9.17	119-138
13	999.27	1996.159	(G) LGPGVGVAPGVGVVPGVGVVPGVG(V)	11.87	499-522
14	585.50	1169.631	(G)GVLGAGQPFPIGG(G)	8.02	696-708
15	778.62	1555.827	(G)AVPGGVPGGVFFPGAGLG(G)	10.09	32-49
16	1571.02	3140.819	(G)LGPGVGVAPGVGVVPGVGVVPGVGVAPGIGLGPG	14.06	499-536
			GVIG(A)		
17	628.05	1254.705	(G)GLGVSTGAVVPQLG (A)	7.58	125-138
18	484.49	967.557	(G)GLGAVPGAVGLG(G)	9.07	665-676
19	422.49	843.436	(G)AGQPFPIGG (G)	5.78	700-708
20	990.19	1979.092	(G) GIGGVGGLGVSTGAVVPQLGAGVG(A)	10.10	119-142
21	676.06	1350.775	(G)GLGVGGLGAVPGAVGLG (G)	9.06	660-676
22	599.56	1197.684	(G)LGVSTGAVVPQLG(A)	7.85	126-138
23	413.38	825.483	(G)AGVLPGVGVG(G)	6.66	281-290
24	441.91	882.204	(G)GAGVLPGVGVG(G)	6.46	280-290
25	1137.82	2275.256	(G) AGVPGPGAVPGTLAAAKAAKFGPGGVG(A)	11.09	590-616
26	719.60	1437.821	(G)ARFPGIGVLPGVPTG(A)	8.83	169-183
27	893.2	1786.908	(S)AGKAGYPTGTGVGPQAAAAAA(K)	19.8	250-270

Table S3. The determined molecular masses and sequences of the 27 peptides released from bovine elastin fibers by pseudoalterin.

a The sequence of each peptide in black was determined by liquid chromatography-mass spectrometry and MASCOT MS/MS Ion Research tools. The left bracketed residue of each peptide indicates the P1-site residue of the left cleavage site. The right bracketed residue of each peptide indicates the P1'-site residue of the right cleavage site.

Amino acid	P4	P3	P2	P1	P1'	P2'	P3'	P4'
Gly	11	17	3	31	14	12	13	15
Ala	6	4	5	1	11	5	2	2
Ser	1	0	0	0	0	0	1	1
Gln	0	3	0	0	0	0	1	0
Pro	6	2	2	0	0	1	6	4
Val	3	4	9	0	2	7	7	5
Leu	3	0	6	0	4	5	1	2
Lys	0	1	0	0	1	0	0	2
Arg	0	0	0	0	0	1	0	0
Phe	2	0	2	0	0	0	1	0
Thr	0	0	1	0	0	0	0	0
Ile	0	1	4	0	0	1	0	1

Table S4. Specificity matrix of pseudoalterin to bovine elastin fibers based on 32 cleavage sites.







B











F

Fig. S9. Analysis of the degradation of the synthetical peptides hydrolysates with pseudoalterin. The molecular masses and sequences of all the peptides were analyzed by LC/MS and FindPept Tool at expasy website, respectively. A, IGGGAGG; B, GIGLGP; C, PGAGARF; D, GVGVAP; E, AAAKAA; F, AAAAAKAAAK.

Strains	Genome	Location	Accession No. of proteases in
	No.		M23
Polaromonas sp. JS666 (1)	CP000316	Sed^b	ABE46000.1, ABE42783.1
Roseobacter denitrificans OCh 114 (2)	CP000362	Australia sed	ABG30058.1
Nautilia profundicola Am-H (3)	CP001279	Ven ^c	ACM93731.1, ACM93477.1
Nitratiruptor sp. SB155-2 (4)	AP009178	Ven in Iheya	BAF69457.1
Sulfurovum sp. NBC37-1 (5)	AP009179	Ven in Iheya	BAF72920.1, BAF72695.1
Thermotoga maritima MSB8 (6)	AE000512	Sed near Vulcano	AAD36886.1
Persephonella marina EX-H1 (7)	CP001230	Ven in Pacific	ACO04799.1, ACO03251.1
Candidatus Ruthia magnifica Cm (8)	CP000488	Ven	ABL02478.1, ABL01827.1
Thiomicrospira crunogena XCL-2 (9)	CP000109	Ven	ABB41394.1, ABB40948.1
Alcanivorax borkumensis SK2 (10)	AM286690	Sed in North Sea	CAL17579.1, CAL16054.1,
			CAL16619.1, CAL17648.1
Desulfobacterium autotrophicum HRM2 (11)	CP001087	Mediterranean sea sed	ACN14260.1
Hahella chejuensis KCTC 2396 (12)	CP000155	Cheju Island sed	ABC28216.1, ABC28556.1,
			ABC28713.1, ABC29831.1,
			ABC31053.1, ABC31896.1,
			ABC32524.1, ABC32886.1,
			ABC32948.1, ABC33568.1,
			ABC29581.1
Shewanella sediminis HAW-EB3 (13)	CP000821	Sed in Halifax Harbor	ABV39073.1, ABV36423.1,
			ABV35714.1
Shewanella halifaxensis HAW-EB4 (14)	CP000931	Sed in Emerald Basin	ABZ77255.1, ABZ75798.1,
			ABZ74618.1
Shewanella sp. W3-18-1 (15)	CP000503	Sed in Pacific Ocean	ABM25854.1, ABM26841.1,
			ABM24560.1, ABM24105.1,
			ABM25390.1
Shewanella piezotolerans WP3 (16)	CP000472	Sed in Pacific	ACJ29884.1, ACJ29020.1,
			ACJ28150.1
Salinispora arenicola CNS-205 (17)	CP000850	Tropical sed	ABW00492.1, ABV99987.1,
			ABV99986.1, ABV98343.1,
			ABV97281.1, ABV97111.1,
			ABV96162.1
Idiomarina loihiensis L2-TR (18)	AE017340	Lo'ihi Seamount ven	AAV81179.1, AAV83434.1,
			AAV82097.1, AAV81287.1,
			AAV81075.1
Shewanella loihica PV-4 (19)	CP000606	Naha ven	ABO24321.1, ABO23341.1,
			ABO23082.1
Oceanobacillus iheyensis strain HTE831 (20)	BA000028	Iheya deep sea mud	Not found
Geobacillus kaustophilus strain HTA426 (21)	BA000043	Mariana Trench sed	Not found
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Table S5. M23 family proteases^a predicted from marine sediment and hydrothermal vent bacteria

a The proteases were all secretary proteins predicted with SignalP 3.0 (possibility >0.5).

b Sed, sediment.

c Ven, hydrothermal vent.

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