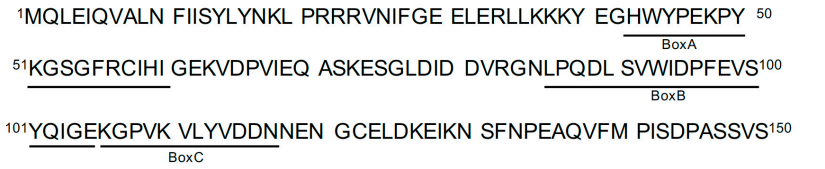
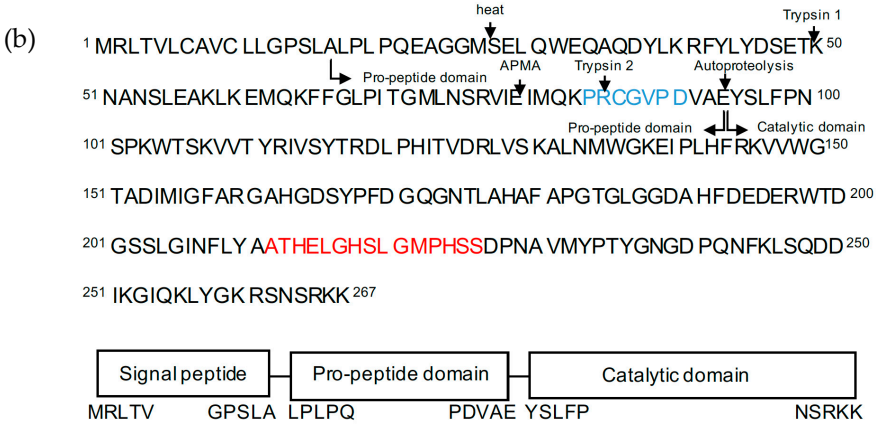
Supplementary1

(a)



(b)



Supplementary data 1 Amino acid sequences of Tob1 and MMP-7

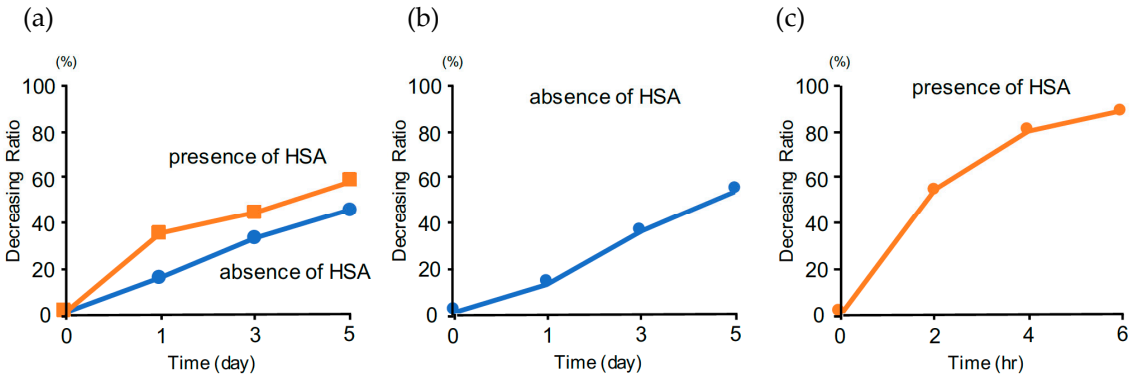
(a) Amino acid sequence of Tob 1

Three domains, BoxA, BoxB and BoxC, are highly conserved in the Tob/BTG family proteins.

(b) Amino acid sequence and domain structure of MMP-7

MMP-7 is the smallest molecule in the family and consists of a signal peptide, pro-peptide domain and catalytic domain. The proteolytic activity of proMMP-7 was controlled by the cysteine-switch mechanism in the non-active form. This mechanism disappeared after cleavage of the prodomain region of proMMP-7 by heat, trypsin, APMA, and the active MMP-7 was produced from the cleavage by auto-catalysis.

Supplementary2



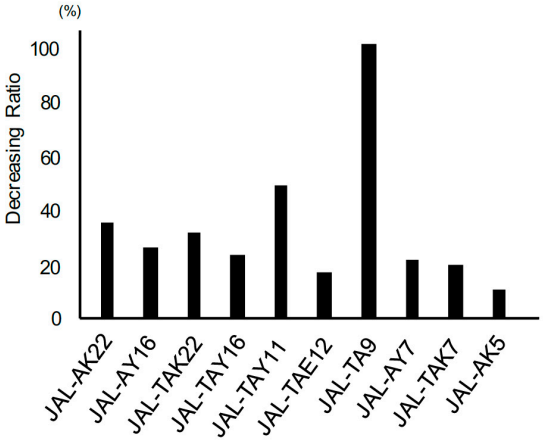
Supplementary data 2 Auto-proteolytic activity of JAL-AK22 and JAL-TA9 in the presence or absence of HSA (a) The auto-proteolytic activity of JAL-AK22 was determined by incubation at 37 °C in the presence or absence of human serum albumin (HSA) in PBS. After incubation for 1, 3 or 5 days, the reaction mixture was analyzed by HPLC. The amount of JAL-AK22 was plotted as the ratio between the final and original levels. (b, c) The auto-proteolytic activity of JAL-TA9 was determined by incubation at 37 °C in the absence (b) or presence (c) of human serum albumin (HSA) in PBS. The amount of JAL-TA9 was plotted as the ratio between the final and original levels.

Supplementary3

(a)

JAL-AK22	1	K	Y	E	G	H	W	Y	P	E	K	P	Y	K	G	S	G	F	R	C	I	H	I	22
JAL-AY16							7	Y	P	E	K	P	Y	K	G	S	G	F	R	C	I	H	I	22
JAL-TAK22	1	K	Y	E	G	H	W	Y	P	E	K	P	Y	K	G	S	G	F	R	M	I	H	I	22
JAL-TAY16							7	Y	P	E	K	P	Y	K	G	S	G	F	R	M	I	H	I	22
JAL-TAY11													12	Y	K	G	S	G	F	R	M	I	H	I	22
JAL-TAE12											9	E	K	P	Y	K	G	S	G	F	R	M	I	20	
JAL-TA9													12	Y	K	G	S	G	F	R	M	I	20		
JAL-AY7													12	Y	K	G	S	G	F	R	18				
JAL-TAK7													13	K	G	S	G	F	R	M	19				
JAL-AK5													13	K	G	S	G	F	17						

(b)



Supplementary data 3 Structure-activity relationship of JAL-TA9 derivative peptides

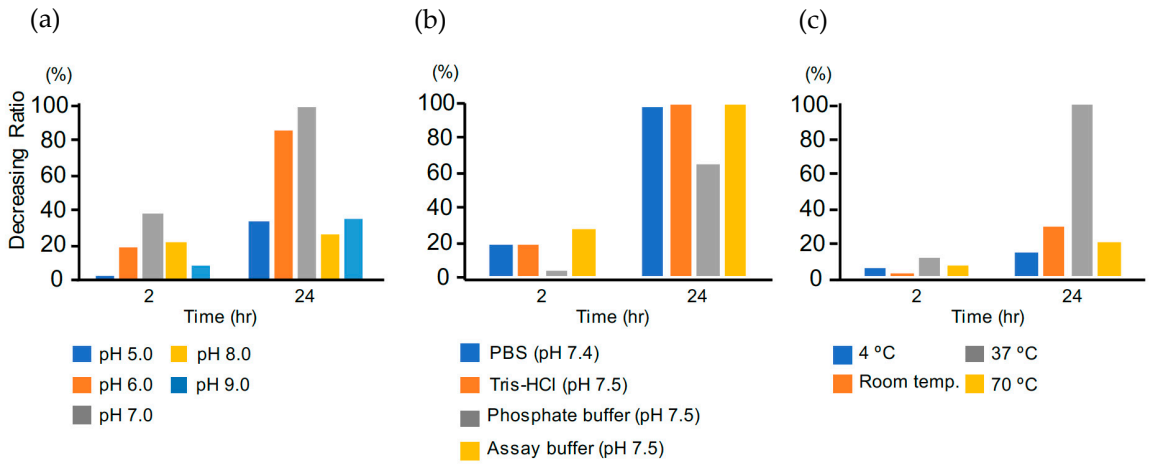
(a) Amino acid sequences of synthetic peptides derived from Tob1 BoxA domain

Our purpose in this study is not to investigate the unknown biological function of Tob1 protein, but to prove the proteolytic activity of synthetic peptide. Thus, we tested the auto-proteolytic activity of various peptides (1a), because we have not found any information about the substrate of the synthetic peptide.

(b) Auto-proteolytic activity of various synthetic peptides

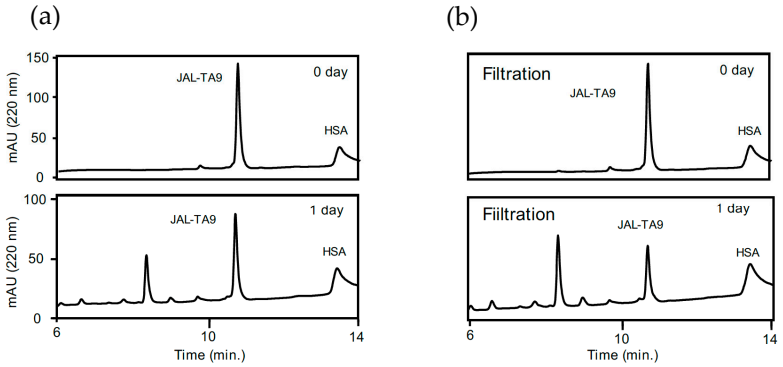
The decreasing ratio was calculated by the comparison of peak heights on HPLC chromatogram on day 0 and day 5. In the results, JAL-TA9, in which Cys residue in nature fragment peptide was substituted to Met residue, showed the most potent auto-proteolytic activity. On the other hand, JAL-AK5, which concluded SG sequence, did not show the potent auto-proteolytic activity. These data indicate that SG sequence is not enough for the auto-proteolytic activity and some secondary structure may be necessary for enzymatic activity.

Supplementary4



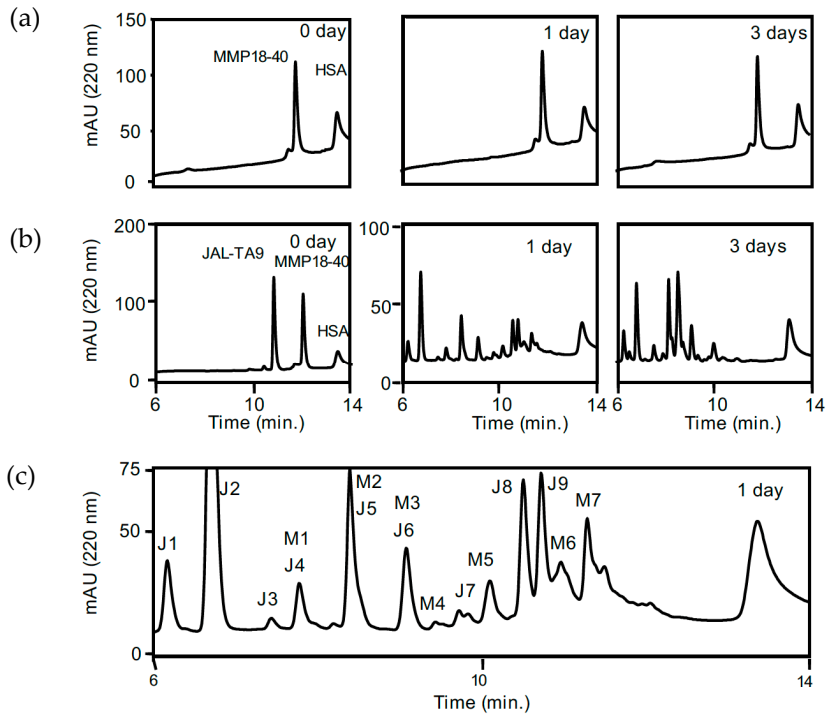
Supplementary data 4 Determination of the optimal conditions for the auto-proteolytic activity of JAL-TA9
The auto-proteolytic activity was examined in the presence of HSA according to the same manner described in Supplementary data 2.
(a) pH dependence
The optimal pH was examined in 200 mM phosphate solution, and JAL-TA9 showed the highest activity at pH 7.0.
(b) Effects of various buffers
JAL-TA9 showed similar auto-proteolytic activity in PBS (pH 7.4), Tris-HCl and assay buffer, which was higher than that observed in Phosphate buffer.
(c) Temperature
The optimal temperature was examined in PBS (pH 7.4), and JAL-TA9 showed the highest auto-proteolytic activity at 37 °C.

Supplementary5



Supplementary data 5 Auto-proteolytic activity of JAL-TA9 with or without filtration
(a) Auto-proteolytic activity of JAL-TA9 without filtration
The reaction mixture of JAL-TA9 was analyzed by an analytical HPLC (upper). The new peaks were identified as the fragment peptides derived from JAL-TA9 after incubation for 1 day (lower).
(b) Auto-proteolytic activity of JAL-TA9 with filtration
The reaction mixture was filtered using MILLEX-GV (Millipore, 0.22um Filter Unit) and TERUMO Syringe (TERUMO, 26G, 1 mL) to remove contaminates and then analyzed by the same manner to the reaction mixture without filtration (upper). The chromatograms obtained after incubation for 1 day were the same to those obtained without filtration (lower). These data proved that the auto-proteolytic activity of JAL-TA9 was not due to contamination of some bacteria.

Supplementary6



Supplementary data 6 Cleavage reaction of MMP-7 fragment peptides by JAL-TA9
(a) Time dependent analysis of MMP18-40
(b) Time dependent analysis of the reaction mixture of JAL-TA9 and MMP18-40
(c) Peak collection of the reaction mixture of JAL-TA9 and MMP18-40

Supplementary data 7 Identification of cleavage sites

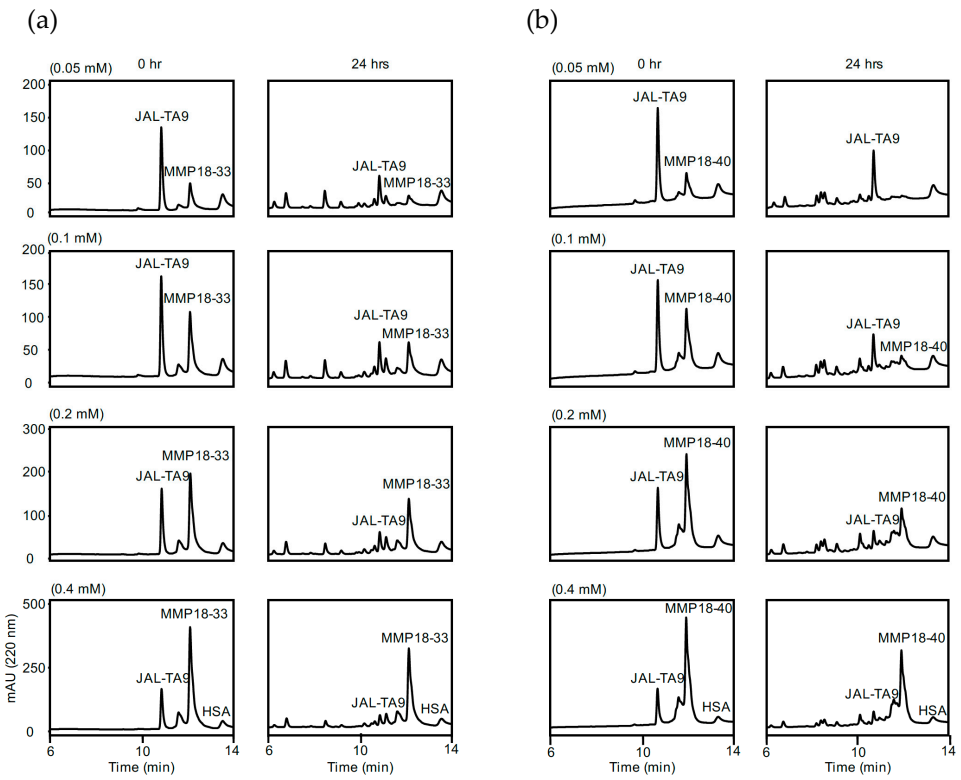
Peak	Fragment	Theoretical MS	Experimental MS
M1	LPQ	356.21	356.2464
M2	AQDY	495.20	494.3074
M3	EQAQDYLK	993.48	933.6076
M4	QAQDYLK	864.43	864.5859
M5	LPLPQ	566.34	566.4081
M5	WEQAQDYLK	1179.56	1179.6898
M6	LPLPQEAG	710.36	709.4516
M6	LPLPQEA	766.42	766.4808
M7	EAGGMSELQWEQAQDYLK	2081.94	2082.1584

Supplementary8

Supplementary data 8 Identification of the complex with JAL-TA9 and AEBSF

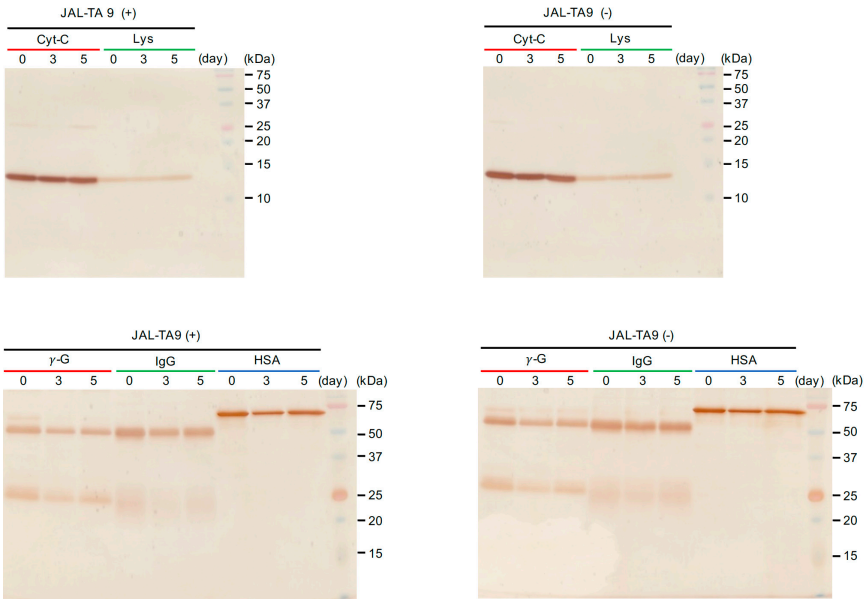
Peak	Fragment	Theoretical MS	Experimental MS
C1	YKGS (AEBSF) GFRMI	1240.76	1240.7322
C2	YKGS (AEBSF) GFRMI	1240.76	1240.7325
C3	MMP 18-40 (AEBSF)	2813.49	2813.6094

Supplementary9



Supplementary data 9 Kinetic parameters
(a) Chromatogram of the reaction mixture of JAL-TA9 and MMP18-33
(b) Chromatogram of the reaction mixture of JAL-TA9 and MMP18-40

Supplementary10



Supplementary data 10 Proteolytic activity of JAL-TA9 against native proteins

First, 0.2 mM JAL-TA9 was incubated with 0.01 % of various proteins at 37 °C in PBS. Next, 10 mL of the reaction mixture was mixed with 2x sample buffer (10 µL) and then boiled for ten min. SDS-PAGE was carried out using 12.5 or 15 % polyacrylamide gel in a Tris-glycine buffer system, followed by silver staining. JAL-TA9 did not cleave g-globin (g-G), rabbit immunoglobulin G (IgG), cytochrome C (Cyt-C), lysozyme (Lys) or human serum albumin (HAS).