SUPPORTING INFORMATION

Figure S1. (**A**) Elution profile from a Sephacryl S-300 chromatography column of Antarctic recombinant pepsinogens. The proteolytic activity in each fraction was measured using denatured hemoglobin as described in "Materials and Methods". Enzyme activity was expressed as ΔOD_{280} ($OD_{280\ [fraction]}$ - $OD_{280\ [blank]}$) after proteolytic digestion. Pepsin activity was determined at pH 2.5 and 37 °C. (**B**) Purification of Antarctic pepsinogens by ion exchange chromatography. The pepsinogen peak from the Sephacryl S-300 column was loaded on a Resource Q column. The column was equilibrated with Tris buffer at a flow rate of 1 mL/min with a linear gradient formed by mixing 10 mL of 20 mM Tris-HCl, pH 8.0 and 10 mL of 500 mM NaCl in Tris buffer. One-milliliter fractions of the eluant were monitored for enzyme activity, absorbance at 280 nm and NaCl concentration. The latter was determined by measuring electrical conductivity. Protease activity was determined as described for Sephacryl S-300. Legend: activity of fish pepsin A1 (▲); activity of fish pepsin A2 (○); continuous line, absorbance at 280 nm; dashed line, NaCl gradient.

Figure S2. Far-UV CD spectra of fish pepsin A1 and A2. CD spectroscopy was performed on homogenous samples of recombinant fish pepsin A1 and A2 (0.1 mg/ml in 2.5 mM sodium acetate buffer, pH 5.3). CD spectra were recorded in the far-UV region (240-200 nm) and each spectrum was averaged 6 times. The results are expressed in terms of residue molar ellipticity. ▲, fish pepsin A1; ○, fish pepsin A2.

Figure S3. Frequency of cleavage at **(A)** the P1 site and **(B)** the P1' site observed after CK-MM digestion by pig pepsin, fish pepsin A1 and fish pepsin A2.

Table S1. Pig pepsin cleavage preference for CK-MM.

Table S2. Fish pepsin A1 cleavage preference for CK-MM.

Table S3. Fish pepsin A2 cleavage preference for CK-MM.

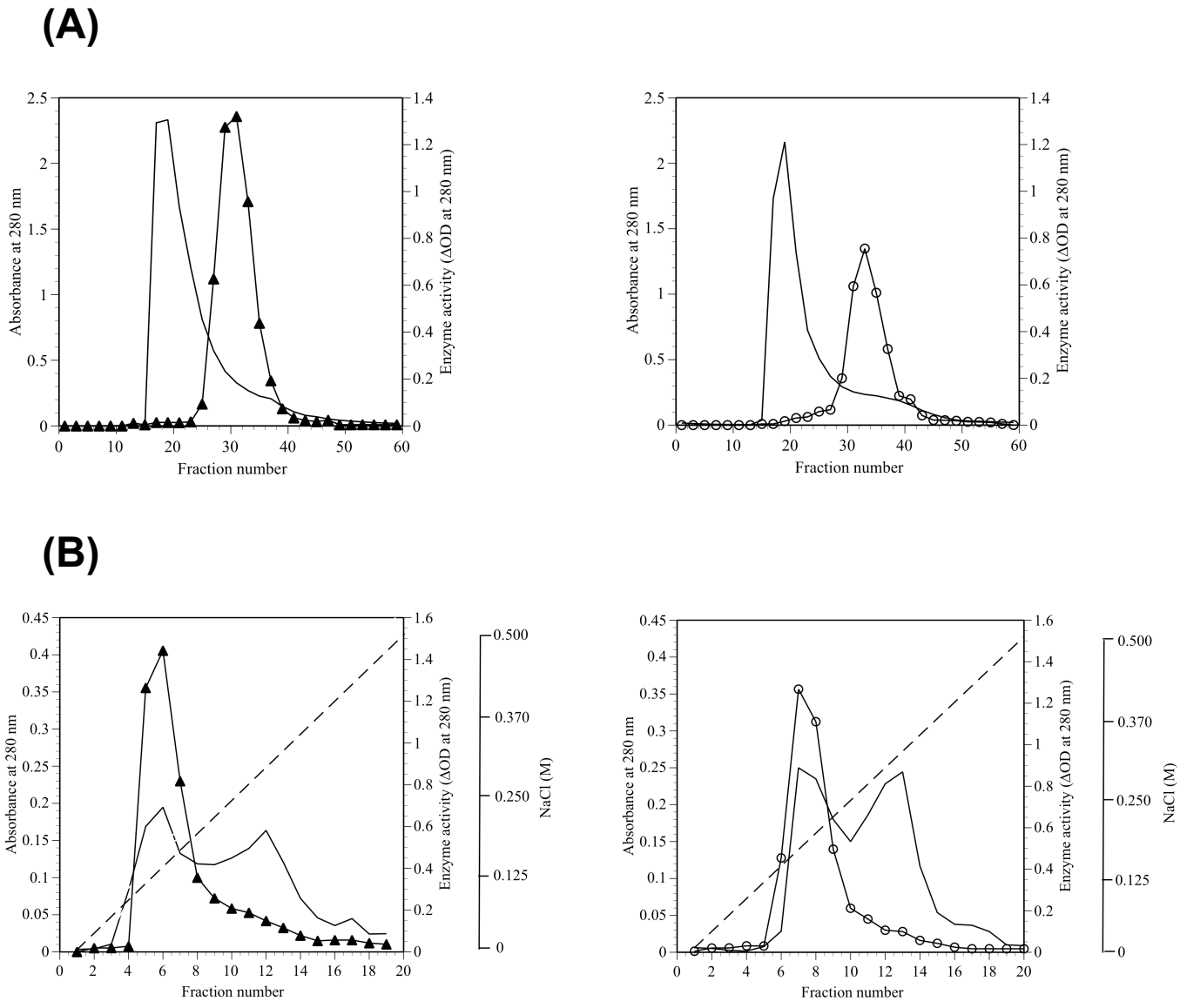

(A)
Absorbance at 280 nm
Enzyme activity (ΔOD at 280 nm)
Fraction number
(B)
Absorbance at 280 nm
Enzyme activity (ΔOD at 280 nm)
NaCl (M)
Fraction number

Figure S1

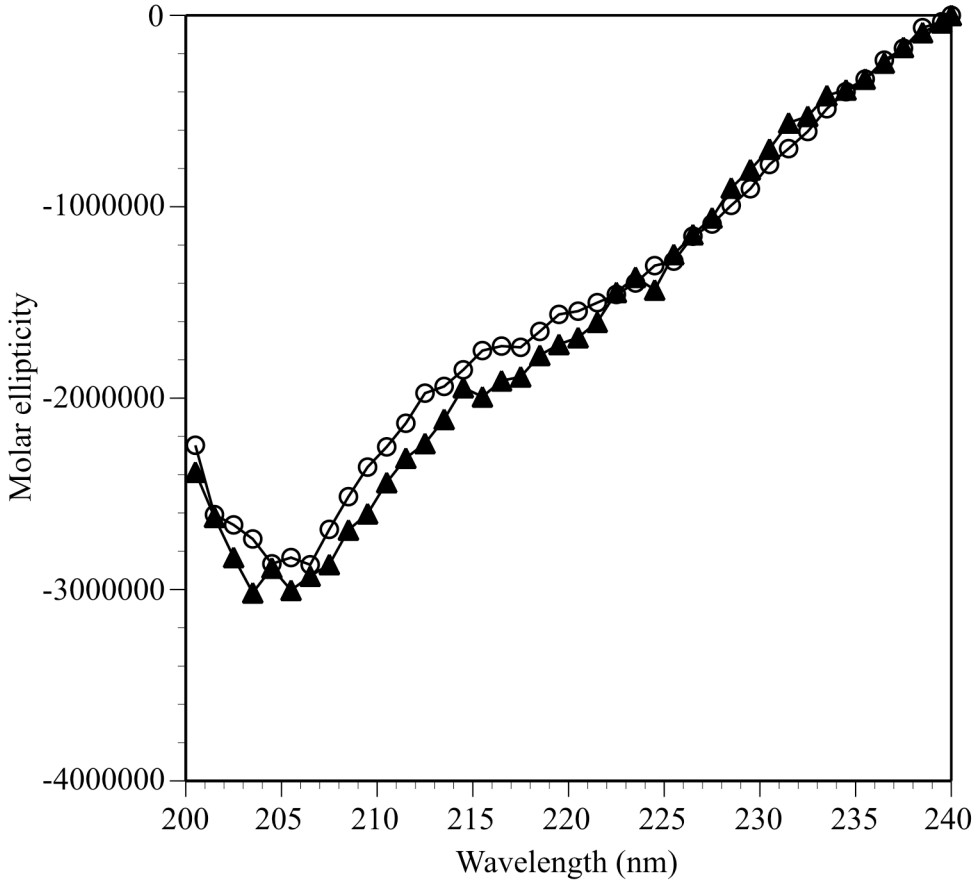
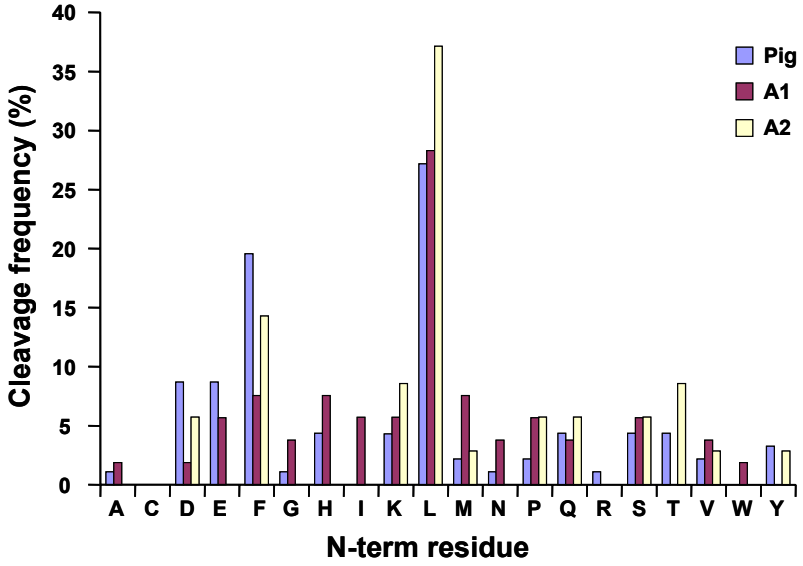

0
-1000000
-2000000
-3000000
-4000000
Molar ellipticity
200
205
210
215
220
225
230
235
240
Wavelength (nm)

Figure S2

(A)


Cleavage frequency (%)
0
5
10
15
20
25
30
35
40
A C D E F G H I K L M N P Q R S T V W Y
N-term residue
Pig
A1
A2

(B)

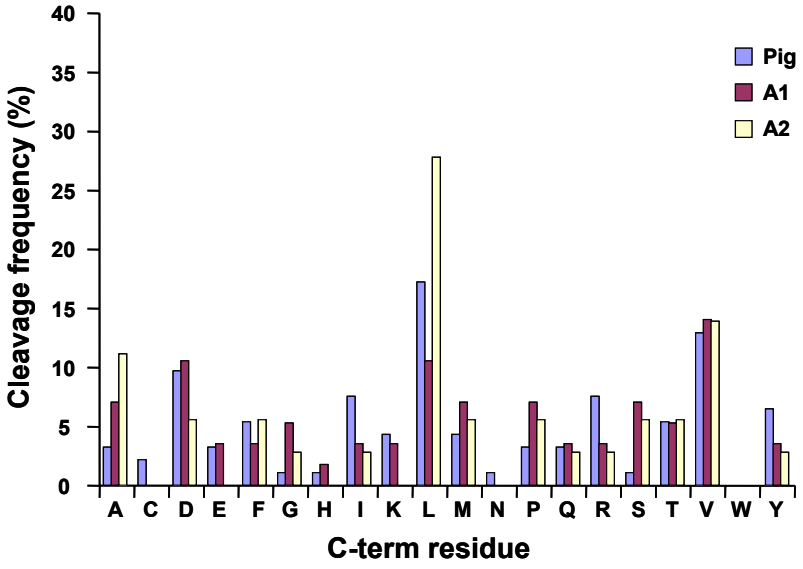

Cleavage frequency (%)
0
5
10
15
20
25
30
35
40
A C D E F G H I K L M N P Q R S T V W Y
C-term residue
Pig
A1
A2

Figure S3

Amino acid position			
P1	Frequency (%)	P1’	Frequency (%)
Ala	1.1	Ala	3.2
Arg	1.1	Arg	7.5
Asn	1.1	Asn	1.1
Asp	**8.7**	**Asp**	**9.7**
Gln	4.3	Cys	2.2
Glu	**8.7**	Gln	3.2
Gly	1.1	Glu	3.2
His	4.3	Gly	1.1
Leu	**27.2**	His	1.1
Lys	4.3	Ile	7.5
Met	2.2	**Leu**	**17.2**
Phe	**19.6**	Lys	4.3
Pro	2.2	Met	4.3
Ser	4.3	Phe	5.4
Thr	4.3	Pro	3.2
Tyr	3.3	Ser	1.1
Val	2.2	Thr	5.4
		Tyr	6.5
		Val	**12.9**

$$Frequency = \frac{Number\ of\ \ cleavages\ observed\ at\ the\ considered\ residue}{Total\ number\ of\ \ cleavages}$$

Table S1

Amino acid position			
P1	Frequency (%)	P1’	Frequency (%)
Ala	1.9	Ala	7.0
Asn	3.8	Arg	3.5
Asp	1.9	**Asp**	**10.5**
Gln	3.8	Gln	3.5
Glu	5.7	Glu	3.5
Gly	3.8	Gly	5.3
His	**7.5**	His	1.8
Ile	5.7	Ile	3.5
Leu	**28.3**	**Leu**	**10.5**
Lys	5.7	Lys	3.5
Met	**7.5**	Met	7.0
Phe	**7.5**	Phe	2.5
Pro	5.7	Pro	7.0
Ser	5.7	Ser	7.0
Tyr	1.9	Thr	5.3
Val	3.8	Tyr	3.5
		Val	**14.0**

Table S2

Amino acid position			
P1	Frequency (%)	P1’	Frequency (%)
Asp	5.7	**Ala**	**11.1**
Gln	5.7	Arg	2.8
Leu	**37.1**	Asp	5.6
Lys	**8.6**	Gln	2.8
Met	2.9	Gly	2.8
Phe	**14.3**	Ile	2.8
Pro	5.7	**Leu**	**27.8**
Ser	5.7	Met	5.6
Thr	**8.6**	Phe	5.6
Tyr	2.9	Pro	5.6
Val	2.9	Ser	5.6
		Thr	5.6
		Tyr	2.8
		Val	**13.9**

Table S3