

Novel pseudo-aspartic peptidase from the midgut of the tick *Rhipicephalus microplus*.

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Supplementary data

Material and Methods

Purification of antibodies anti-RmPAP from rabbit sera: Purified recombinant RmPAP_{WT} was adsorbed in CnBr-Activated Sepharose 4B (GE Healthcare) following manufacturer's instructions. Sera from rabbits immunized against RmPAP_{WT} were dialyzed with PBS and applied to the RmPAP_{WT}-sepharose resin. Antibodies elution was performed with KCl 0.1 M pH 2.0 in fractions of 1 mL containing 0.1 mL of Tris-HCl 1 M pH 7.5 for pH neutralization. The fractions 18 – 21 were pooled, dialyzed with PBS and concentrated using an Amicon (10 kDa) (Merck) by centrifugation (15 min, 2500 x g at 4°C). Purified antibodies were stored at -20°C prior to use.

Table 1: Primers sequences

	Used in	Restriction sites	Sequence (5' – 3')
RmPAP.FW	pET14b	Xho I	CCGCTCGAGCCACTGGACAACACTACAACA ATC
RmPAP.RV		Bpu1102	TAGTTATTGCTCAGCCTATTTTCGCGATG GCGAAACC
qPAP.FW	qPCR	-	GGATGGAAAATACCAACTGCATAC
qPAP.RV		-	TCTTGTCCGCCAAATATGAAC
qELF1a.FW		-	CGTCTACAAGATTGGTGGCATT
qELF1aRV		-	CTCAGTGGTCAGGTTGGCAG
RmPAP.RNAI. FW	dsRNA	-	GGATCCTAATACGACTCACTATAGGGAA TTCTTGGTCATGCTT

RmPAP.RNAI. RV		-	GGATCCTAATACGACTCACTATAGGGGC GGGTCCCCCAATGAA
GFP.RNAi.FW		-	GGATCCTAATACGACTCACTATAGGGTG TTCAATGCTTTGCGAGA
GFP.RNAi.RV		-	GGATCCTAATACGACTCACTATAGGAAA GGGCAGATTGTGTGGAC
RmPAP.MUT. FW	Mutation	-	GCTCGGCCGGCTGACACGGAG
RmPAP.MUT. RV		-	CTCCGTGTCAGCCGGCCGAGC

Table 2: Titration of phages selection with rRmPA

Rounds	Total entry phages	Total output phages	% recovered phages	Enrichment
1	2.09x10 ¹⁰	8.55x10 ⁴	1.03x10 ⁻⁵	-
2	9.75x10 ⁸	1.36x10 ⁵	1.39x10 ⁻⁴	34.0
3	2.40x10 ⁸	3.98X10 ⁴	1.66X10 ⁻⁴	1.19

	10	20	30	40	50	60
1	ATGCCGATGCTCAGGCTAAGCCTCTTTGTTTTATTGGGATGGGCAGGACCTTGTCTGGCC					
1	M P M L R L S L F V L L G W A G P C L A					
	70	80	90	100	110	120
61	CGTGGCTCAAGTAATGGCCCTGCCGGTCAAGCACTCAAAATGGTGTCTGGTACCACTGGAC					
21	R G S S N G P A G Q A L K M V S V P L D					
	130	140	150	160	170	180
121	AACTACAACAATCTCACATACATCACAAAGCTCATGCTAGGTACGCCACCGCAGAAATTC					
41	N Y N N L T Y I T K L M L G T P P Q K F					
	190	200	210	220	230	240
181	TTGGTCATGCTTGATTCTGGCGGGTACAACGTGGGTTCTGCCTACTACTGCACGCAAGCG					

61 L V M L **D** S A G T T W V P A Y Y **C** T Q A
 250 260 270 280 290 300
 241 GAGCCCTGCTTTGAACACAAGATGTATGACTGGAGCAAGTCGACGACGCAGAGCAGTGAC
 81 E P **C** F E H K M Y D W S K S T T Q S S D
 310 320 330 340 350 360
 301 TGGGAAGACGGTGCCACACCCTTTGGAAGGGGCAACGTAACCGGCTACGTATTTCTGGGAT
 101 W E D G A T P F G R G N V T G Y V F R D
 370 380 390 400 410 420
 361 GCTCATGAGCTCGGCGGCGTGTCCCTCGGAAATCTTCAGTTTGTGAATGGAATAGGCTTC
 121 A H E L G G V S L G N L Q F V N G I G F
 430 440 450 460 470 480
 421 CCCGGGGTGCCAGCTTCGTGGACAAGCCCTTCGACGGCGTCTTCGGGCTGCGTCTCGGC
 141 P G V P S F V D K P F D G V F G L R L G
 490 500 510 520 530 540
 481 GCAAACCTCTACGCTTTACGAAATGGCTAAATCGGGTGTAATCGGCAGTCCAAAGGTGGGA
 161 A N S T L Y E M A K S G V I G S P K V G
 550 560 570 580 590 600
 541 CTTTATTTTAGCAACGACATGACCACTCCAGGGGAAGCCCTCTTCGGAGGTGCAAATGAG
 181 L Y F S N D M T T P G E A L F G G A N E
 610 620 630 640 650 660
 601 CAGCACTACCAAGGTTCAATGACCTTCATGGATGCTCTCGACAGTGCCTTTAAGGTTTCAT
 201 Q H Y Q G S M T F M D A L D S A F K V H
 670 680 690 700 710 720
 661 CTAAATGGATATGAAGTGGCTGGTCAGAGAGAAAGGCTACGAGCATCGTGGGCTCGGCCG
 221 L N G Y E V A G Q R E R L R A S W A R P
 730 740 750 760 770 780
 721 GCTCCAACGGAGCCCTTCATTGGAGGACCCGCGAGCGAAATAAAGAAAATCAACACACTG
 241 A P T E P F I G G P A S E I K K I N T L
 790 800 810 820 830 840
 781 CTTGGTGCCAGGAAGATCGAGGATGGAAAATACCAACTGCAGTACGAGCTCCACTGCAAC
 261 L G A R K I E D G K Y Q L Q Y E L H **C** N
 850 860 870 880 890 900
 841 GCCAGGACTTCCGACTTTACGTTTCATATTTGGCGGACAAGAGATCGTCCTGAGGCCAGAA
 281 A R T S D F T F I F G G Q E I V L R P E
 910 920 930 940 950 960
 901 GACTACGTGGTGAAGGTGGAGACAACAATAAACTACATGTTACAGCGGCTTCATTGAA
 301 D Y V V K V E T T T K T T **C** Y S G F I E
 970 980 990 1000 1010 1020
 961 GCGGAAGAGGTGGCGAATGCCAGTTGGCACTTAGGTCTTGTATTCTCAGACGAGTCTAC
 321 A E E V A N A S W H L G L V F L R R V Y
 1030 1040 1050 1060 1070 1080

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1021      ACCGTGCTCGAGGCGCCGCTAGAGCCCTCTGGTAATGGACGAGTGGGTTTCGCCATCGCG
341      T V L E A P L E P S G N G R V G F A I A

1081      AAATAA
361      K  *

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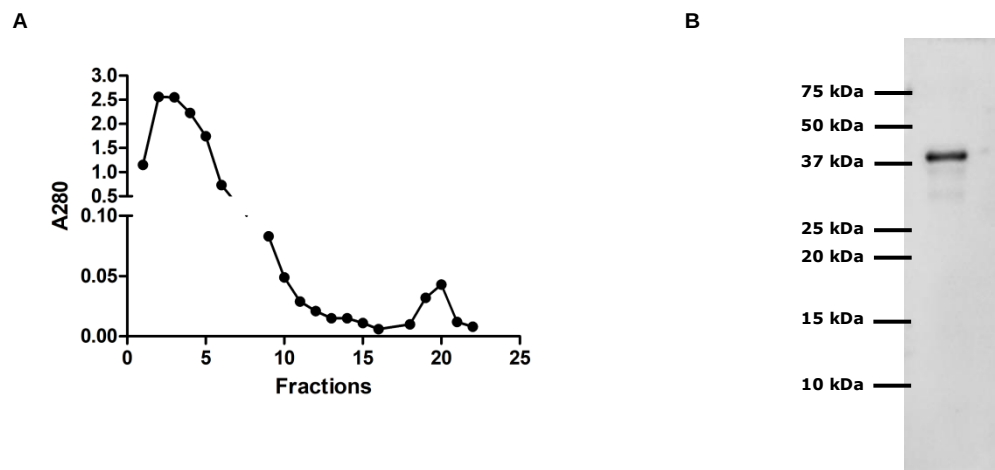
Sup. Figure 1: Complete nucleotide and translated amino acid sequence of RmPAP amplified from the cDNA library of *Rhipicephalus microplus* midgut. Putative signal peptide is underlined, the Cys residues are in gray boxed and the first Asp residue related to the proteolytic activity is in red.

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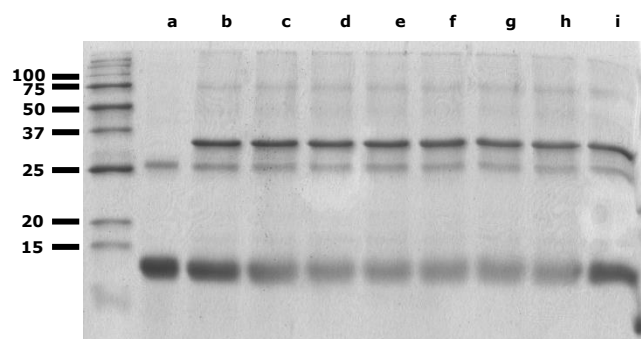
                        10          20
                .....|.....|.....|.....|.....|....
WT      RLRASWARPAPTEPFIGGPASEIKKINT
MUT     RLRASWARPADTEPFIGGPASEIKKINT

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Sup. Figure 2: Alignment of RmPAP Pro²⁴² and Asp²⁴² amino acid sequence in the mutated region.

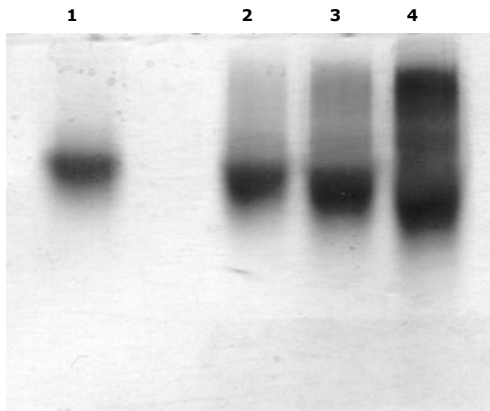


Sup. Figure 3: (A) Purification of anti-RmPAP antibodies using RmPAP-Sepharose CnBr-4B resin. Resin was equilibrated with PBS and 1.0 mL of rabbit sera immunized against rRmPAP was applied to the column, elution was carried with 0.2 M KCl pH 2.0 and pH neutralization was made by the addition of 0.1 mL of 1.0 M Tris-HCl pH 8.0. Fractions 18 – 21 were pooled, concentrated and used for (B) western blot assays with rRmPAP.

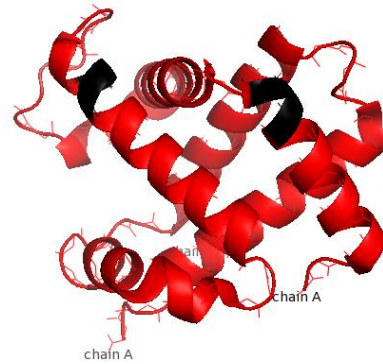


Sup. Figure 4: SDS-PAGE 15% containing the (a) bovine hemoglobin and RmPA_{MUT} with bovine hemoglobin incubated in different pH (b) 2.5, (c), 3.0, (d) 3.5, (e) 4.0, (f) 4.5, (g) 5.0, (h) 5.5 and (i) 6.0.

A



B



Sup. Figure 5: (A) Native PAGE 12% containing the (1) bovine hemoglobin, (2) bovine hemoglobin:RmPAP_{MUT} (1:1), (3) bovine hemoglobin:RmPAP_{MUT} (1:5), and (4) bovine hemoglobin:RmPAP_{MUT} (1:10). (B) Bovine hemoglobin chain A crystal structure (pdb: 2QSP) with VKG (GKV) regions highlighted in black.