

Supplementary information

Convergent evolution of plant and animal embryo defences by hyperstable non-digestible storage proteins

María Yanina Pasquevich, Marcos Sebastián Dreon, Jian-Wen Qiu, Huawei Mu and
Horacio Heras

Supplementary Table 1

Supplementary Table 1 | *In silico* digestion of PmPV1 subunits and BSA by the enzymes assayed in the *in vitro* experiments. Values represent the number of possible cutting sites in the subunits. No missing cutting sites were allowed. Values between brackets are the number of cutting sites normalized for a 20 kDa subunit to highlight the similarity among them and the control protein.

	Mw (KDa) ^a	Pepsin	Trypsin	Elastase ^b	Chymotrypsin	Proteinase K
PmPV1-1*	23.184	70 (60)	26 (22)	86 (74)	16 (14)	80 (69)
PmPV1-2	22.399	74 (66)	22 (20)	100 (89)	12 (11)	81 (72)
PmPV1-4a	21.901	69 (63)	23 (21)	92 (84)	16 (15)	76 (69)
PmPV1-4b	22.269	65 (58)	17 (15)	92 (83)	18 (16)	78 (70)
BSA	69.324	238 (69)	82 (24)	230 (66)	53 (15)	211 (61)

^aCalculated using ExPaSy PeptideMass (Wilkins *et al.* 1997). ^bCalculated using MS-Digest

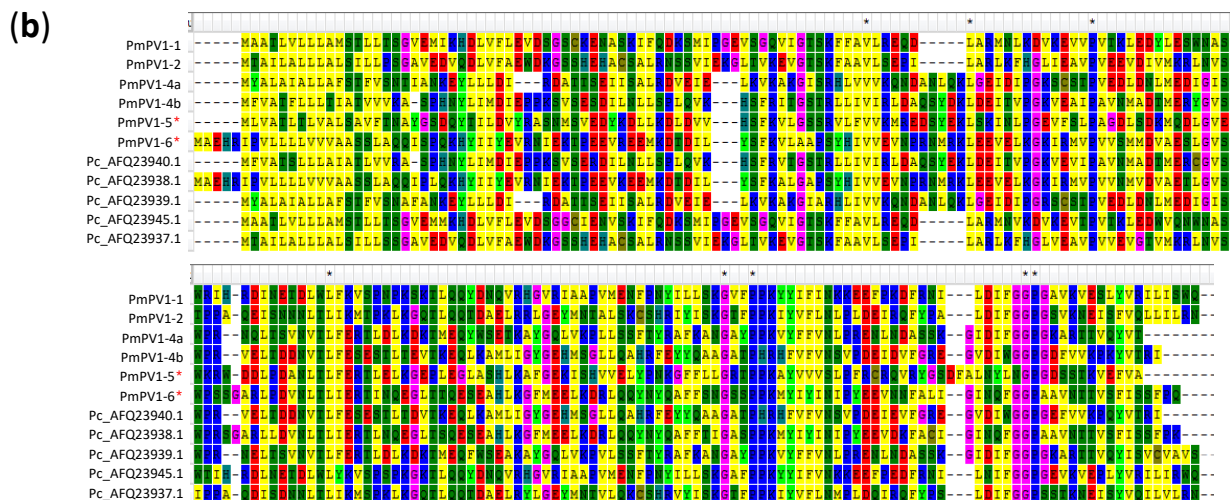
(<http://prospector.ucsf.edu>) *Subunits named according to Pasquevich *et al.* 2014

Supplementary Results

Supplementary Figure 1 | Sequences of PmPV1 subunits and PmPV1 and PcOvo sequence alignment. (a) Alignment of deduced aminoacid sequences of PmPV1 subunits. A conserved sequence is marked in red. The putative signal sequences are in italics. Potential phosphorylation sites are underlined, and potential N-glycosylation sites (NXS/T) are underlined and in bold. N-Glycosylation sites (NXS/T) with low probability of being glycosylated are not shown. *: fully conserved site. (b) PmPV1 and PcOvo sequences alignment. Alignment was done using MEGA 6. Pc and Pm prefixes indicate *P. canaliculata* and *P. maculata*, respectively. Sequence AFQ23939.1 has been trimmed at amino acid 217. * indicate potential subunits.

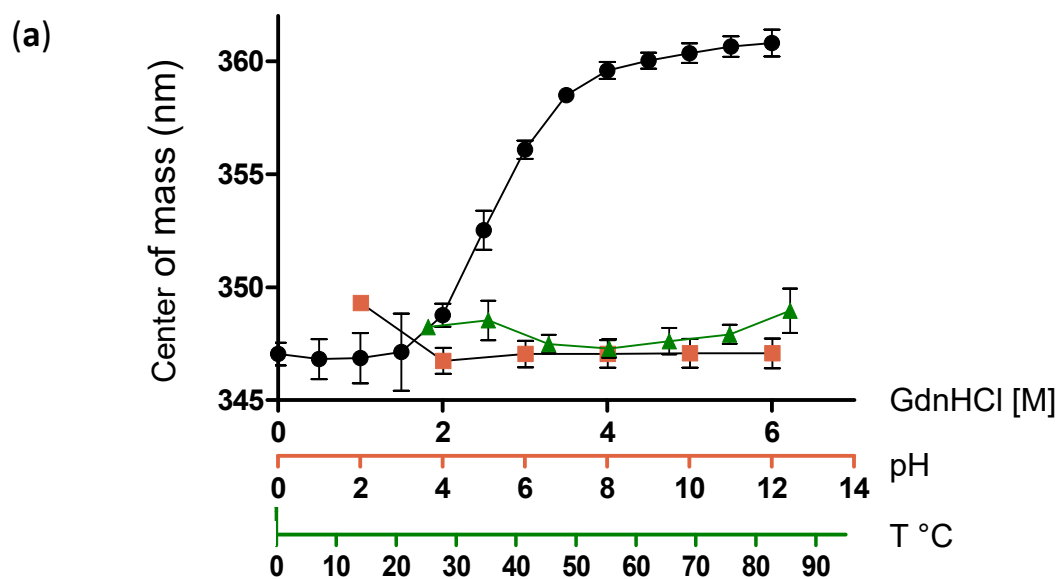
(a)

PmPV1-1	-----MA-ATLVLLLAMSTLLTSGVEMIKHDLVFLEVDS--GSCKEN NASK IFQDKSMIPG
PmPV1-2	-----MT-AILALLLALSILLPSGAVEDVQDLVFAEWDK--GSSHEHAC SALR NSS VIEK
PmPV1-6*	MAEHRIPVLLLLVVVAASSLAQQISPQK--HYIIYEVRNI-EKTPEEVREEMK---DTDI
PmPV1-5*	-----ML-VATLTLVALSAVFTNAYGSD--QYTILDVYRAS NMS VEDYKDLLK---DLDV
PmPV1-4a	-----MY-ALAIALLAFSTFVSNTIANK--EYLLLDIRDA---TTSEIISALR---DVEI
PmPV1-4b	-----MF-VATFLLLTATV-VKASPH--NYLIMDIEPPKSV SES DILNLLS---PLQV
	: : : : . : : : . . . :
PmPV1-1	EVSGQVIGTSKFFAVLRE-----QDLARMNLKDVKEVVPVTKLEDYLESWNASWRIH-RD
PmPV1-2	GLTVKEVGTSKFAAVLSE-----PILARLKFHGLIEAVPVEEVDIVMKRL NVS TPPA-QE
PmPV1-6*	LYSFKVLAAPSYHIVVEVNPRNMRKLEEVELKGIKRMVPPVSMMDVAESLGVSWPSSGAR
PmPV1-5*	VHSFKVLGSSRVLFVVKMREDSY EKL SKINLPGEVFSLPAGDLSDKMQDLGVWEKRW-DD
PmPV1-4a	ELKVKAKGISRHLVVVKQNDANLQKLGEIDIPGKSCSTPVEDLDNLMEDIGISWPRN--Q
PmPV1-4b	KHSFRITGSTRLLIVIRLDAQSYDKLDEITVPGKVEAIPAVNMADTMERYGVSWPRV--E
	. : . * : * . : . * . : : . .
PmPV1-1	INETDLWLFKVSPNPKSKTLQQYDNQVRHGVRIAAPVMENFPNYILLSKGVFPPKYYIFI
PmPV1-2	ISNN NLT LIIKMTPKLKGQTLQQTDAELRRLGEYMNLTALSKCSHRIYISKGTFPKIIYVFL
PmPV1-6*	LPD VNLT LIIERTINQEGLTTQSEAHKGFMEELKDRLQQYNYQAFFS NGS SPPKMYIYI
PmPV1-5*	LPD ANLT LFERTLELKGEPLEGLASHLKAFGEKISHVVELYPNKGFFLLGRTPPKAYVVV
PmPV1-4a	LTSV NVT LFERTLDLKDKTMEQYWSETKAYGQLVKPLLSSTYRAFKANGAYPPKVVYFFV
PmPV1-4b	LTDD NVT LFESESTLTEVTKEQLKAMLIGYGEHMSGLLQAHRFEYYQAAGATPHRHVFV
	: . : : * : : : . : . * * : : . :
PmPV1-1	NKKEEFPK--DF-RNILDIF GGPG AVKVESLYVRILISWQ
PmPV1-2	NLPLDEIR--QF-YPALDIF GGPG SVKNEISFVQLLILRN
PmPV1-6*	NIPYEEVN--NFALIGINQF GGPA AV NNT VSFISSFPQ--
PmPV1-5*	SLPFRRCRQVRYGSDFALNYL NGPG DSSTKVEFVA-----
PmPV1-4a	NLPRENLN--DASSKGIDIF GGPG KARTTVQYVT-----
PmPV1-4b	NSVPDEID--VFGREGVDIW GGPG DFVVKPKYV TRI ----
	. : : . * . : : .

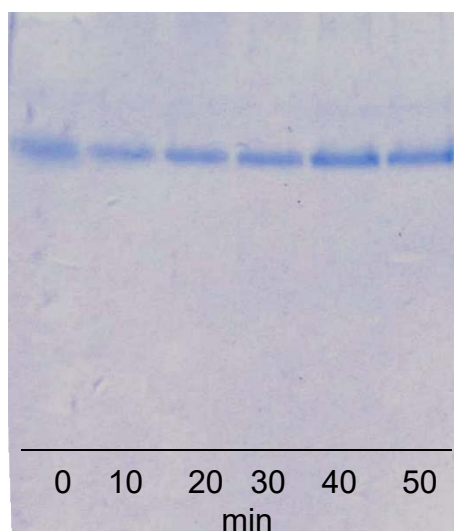


Supplementary Results

Supplementary Figure 2 | Structural stability. (a) Comparison of different denaturant agents on PmPV1 structural stability by fluorescence emission spectroscopy. Black circles: unfolding of PmPV1 population as a function of GdnHCl at pH 7.4 and 25°C; red squares: effect of pH at 25°C; green triangles: effect of temperature at pH 7.4. **(b) Behavior of PmPV1 in native PAGE boiled for 0–50 min.**

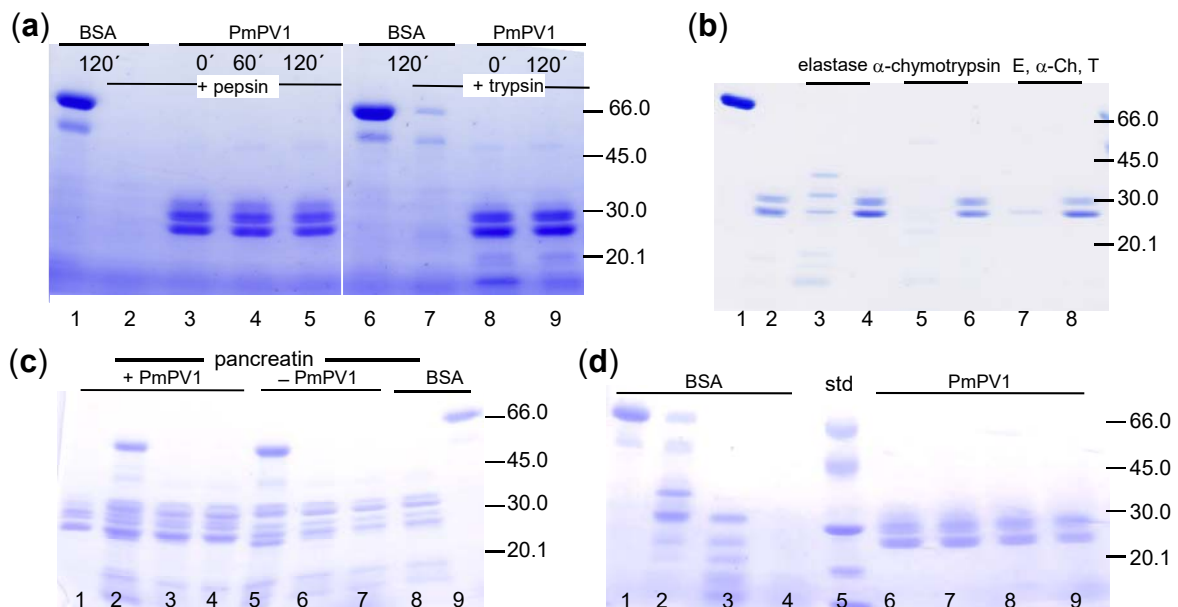


(b)



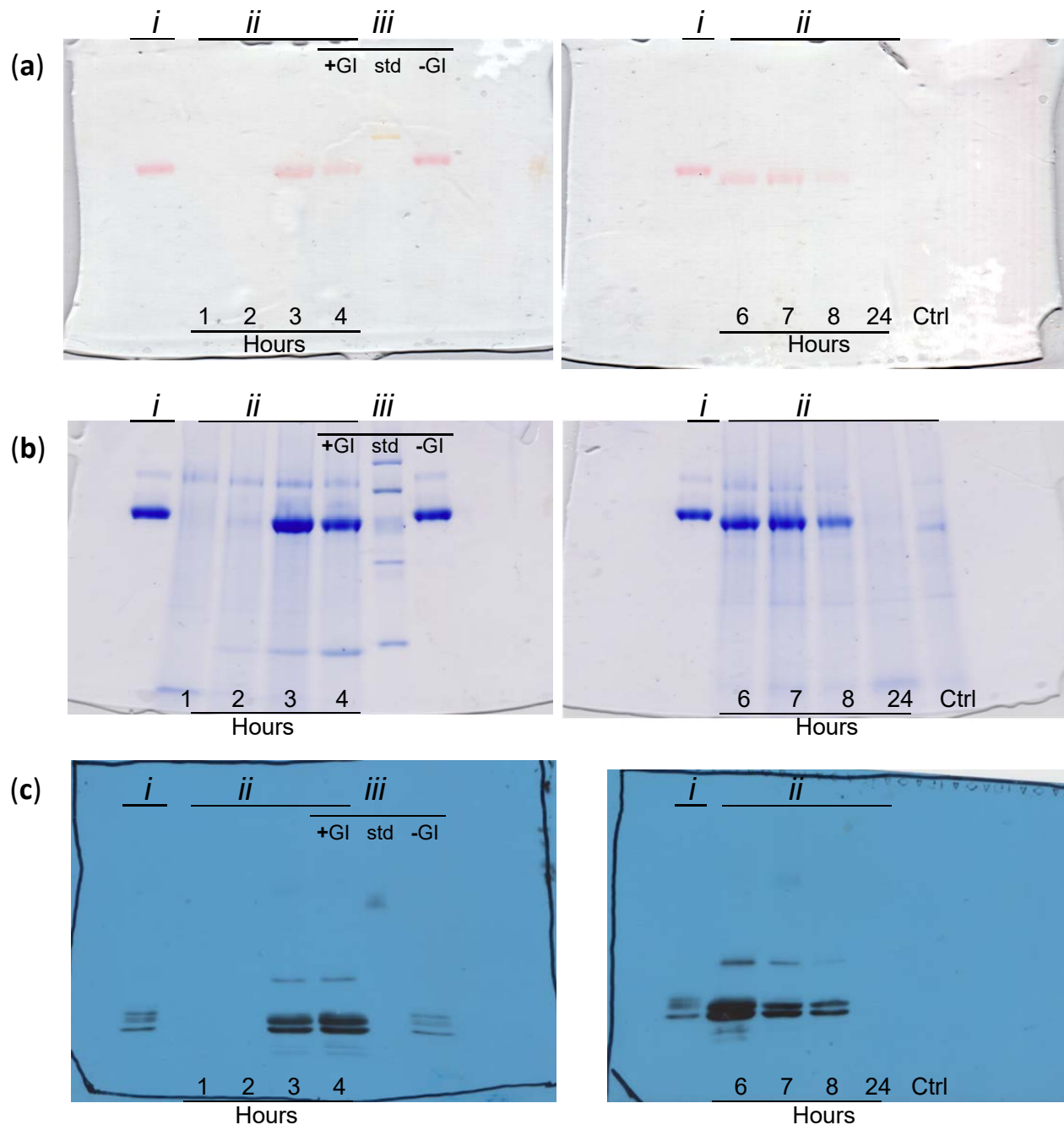
Supplementary Results

Supplementary Figure 3 | Simulated digestion of PmPV1 analyzed by SDS-PAGE. (a) Gastric (lanes 1-5) and duodenal (lanes 6-9) digestion. Lanes 1-2: BSA controls of gastric digestion; Lanes 3-5: PmPV1 after incubation with pepsin for 0, 60 and 120 min. Lanes 6-7: BSA controls of duodenal digestion; lanes 8-9: PmPV1 digested with trypsin for 0 and 120 min, respectively. **(b)** Other duodenal proteases-120 min digestion. Lanes 1-2: BSA and PmPV1 without enzyme; lanes 3-4: BSA and PmPV1 with elastase (E), respectively; lines 5-6: BSA and PmPV1 with α -Chymotrypsin (α -Ch), respectively; lanes 7-8: BSA and PmPV1 with an Elastase, α -Chymotrypsin and Trypsin (T) mixture. BSA in lanes 3, 5 and 7 were used as positive control. **(c)** Pancreatin digestion. Lane 1: PmPV1 without pancreatin; lanes 2-4 PmPV1 with pancreatin immediately added, 60 and 120 min, respectively; lanes 5-7 pancreatin without PmPV1 at 0, 60 and 120 min, respectively; lanes 8-9: positive (BSA + pancreatin) and negative (BSA without pancreatin) controls after 120 min incubation. **(d)**, Proteinase K digestion of PmPV1. Lane 1: Negative control; lines 2-4 BSA with 0, 1, 10, 100 μ g/mL proteinase K; lanes 6-9: PmPV1 with 0, 1, 10, 100 μ g/mL proteinase K. Lane 5 (std): molecular weight markers of 66, 45, 30, 22.1 and 14.4 kDa.



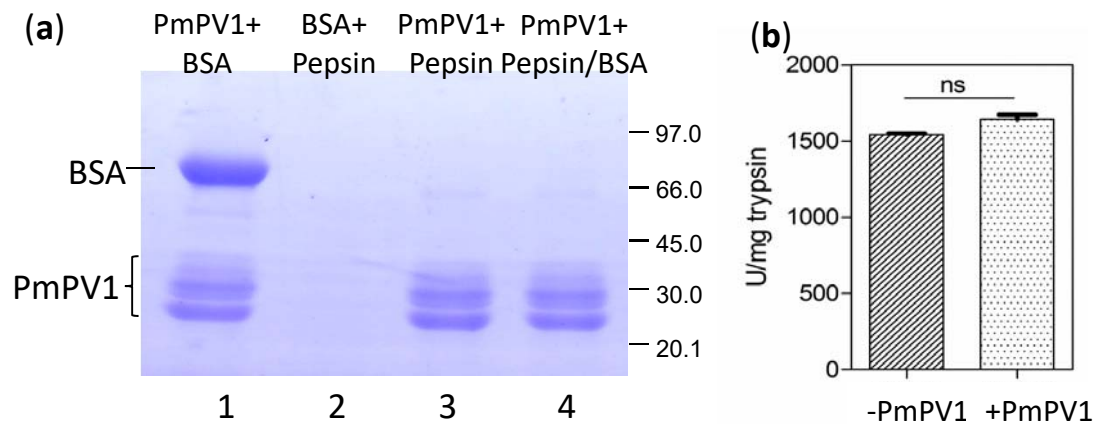
Supplementary Results

Supplementary Figure 4 | *In vivo* digestibility of PmPV1 (a) Native PAGE without staining, showing coloured PmPV1; (b), Coomassie staining of the same gel shown on (a). (c) Western blot of faecal protein antibody known to cross-react with PmPV1 subunits. *i*. Purified PmPV1. *ii*. Faecal proteins showing PmPV1 in faeces collected 1–24 h after first administration. Ctrl: Faecal proteins collected in the control mouse 4 h after beginning the experiment. *iii*. Comparison of PmPV1 with (+GI) or without (-GI) passage through the gastrointestinal tract. Molecular weight marker (std): thyroglobulin (669 kDa), ferritin (yellowish-coloured, 440 kDa), catalase (232 kDa), lactate dehydrogenase (140 kDa), and Albumin (67 kDa).



Supplementary Results

Supplementary Figure 5 | Pepsin and trypsin inhibition capacity of PmPV1. (a) SDS-PAGE showing no pepsin inhibition by PmPV1. Lane 1, PmPV1 and BSA without Pepsin (negative control); Lane 2, BSA incubated with pepsin. Lane 3, PmPV1 incubated with pepsin. Lane 4, BSA after pepsin incubation for 10 min in the presence of PmPV1. (b) Trypsin specific activity without (-PmPV1) and with PmPV1 (+ PmPV1). Ns: not significant ($P>0.05$).



Supplementary Results

Supplementary Figure 6 | Behaviour of PmPV1 with a reducing agent. Dissociating gel electrophoresis (SDS-PAGE) in 4–20% polyacrylamide gradient. Lane 1: Low molecular weight standards; Lane 2: PmPV1 incubated without β -mercaptoethanol; Lane 3: PmPV1 with reducing agent.

