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Structural Properties of Macrodontain I, a Cysteine Protease from *Pseudananas macrodontes* (Morr.) Harms (Bromeliaceae)

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Abstract The primary structure of macrodontain I, a peptidase from *Pseudananas macrodontes* fruits, was determined using Edman's degradation. The enzyme is a non-glycosylated peptidase composed by 213 amino acids with a calculated molecular weight of 23,486.18 Da, pI value 6.99, and a molar extinction coefficient at 280 nm of 61,685 M^{-1} cm^{-1}. The alignment of the sequence of macrodontain I with those cysteine peptidases from species belonging to the family Bromeliaceae showed the highest identity degree (87.74%) against fruit bromelain. A remarkable fact is that all these peptidase sequences show two Met contiguous residues (Met121 and 122) and the nonapeptide VPQSIDWRD located in the mature N-terminal region. Residues Cys26 and His159, which constitute the catalytic dyad in all cysteine peptidases, as well as active site residues Gln20 and Asn176, characteristic of Clan C1A, are conserved in macrodontain I. The 3-D model suggests that the enzyme belongs to the $\alpha + \beta$ class of proteins, with two disulfide bridges (Cys23-Cys63 and Cys57-Cys96) in the α domain, while the β domain is stabilized by another disulfide bridge (Cys153-Cys201). Further, we were able to establish that the cysteine peptidases from *P. macrodontes* are involved in the anti-inflammatory activity.

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Keywords *Pseudananas macrodontes* · Bromeliaceae · Amino acid sequence · Anti-inflammatory activity · Cysteine peptidase · Plant endopeptidase · Macrodontain I

Abbreviations

CNBr	Cyanogen bromide
E64	*trans*-Epoxysuccinyl-L-leucylamido(4-guanidino)butane
HPLC	High-performance liquid chromatography
PVDF	Polyvinylidene fluoride
MALDI-TOF MS	Matrix-assisted laser desorption ionization-time-of-flight mass spectrometry
RP	Reversed phase
TFA	Trifluoroacetic acid

Introduction

Enzymes known as cysteine proteases are often thought of as all being related, but the reality is that they have come from a number of quite separate evolutionary origins [1]. According to the Peptidase Database (MEROPS, release 11.0), cysteine peptidases can be classified in 89 families [2], which can be grouped into 10 clans (CA, CD, CE, CF, CL, CM, CN, CO, CP, and CQ) and five clans of mixed catalytic type (PA, PB, PC, PD, and PE). The C1A subfamily includes papain (type peptidase) and other related plant proteinases, such as chymopapain, stem bromelain, ananain, fruit bromelain, and ficin.

Plants are equipped with a proteolytic machinery that regulates the fate of proteins. This machinery has generally been viewed in a housekeeping role, serving to remove non-functional proteins and to release amino acids for recycling. However, proteases also appear to play key roles in the regulation of biological processes in plants, such as the recognition of pathogens and pests and the induction of effective defense responses [3].

From an industrial point of view, proteolytic enzymes account for approximately 60% of all enzyme sales. They have been widely applied in pharmaceutical, medicinal, food, detergent, leather and biotechnological industries [4, 5].

Pharmacological action of plant cysteine proteinases has been reported in mammalian wound healing, immunomodulation, digestive disorders, inflammation, and antihelmintic and neoplastic treatment [6, 7], as well as in hemostasis [8, 9]. Bromelain, an aqueous extract obtained from stems and immature fruits of *Ananas comosus* L, has demonstrated numerous pharmacological effects related to their cysteine proteinases [10]. As therapeutic, bromelain is very well characterized and most of preclinical and clinical trials investigating the effects of systemic enzyme therapy have been conducted with bromelain or mixtures of enzymes that also contained bromelain [11].

Bromeliaceae, the family of *Ananas comosus*, is a plant family to which belong many species that produce large amounts of proteases. To date, a number of cysteine endopeptidases from species belonging to Bromeliaceae have been isolated and characterized: stem and fruit bromelain (EC 3.4.22.32 and EC 3.4.22.33, respectively), ananain (EC 3.4.22.31), comosain, and a novel aspartic acid protease obtained from *A. comosus* [12–17], pinguinain (EC 3.4.22.33, formerly EC 3.4.99.18), isolated from *Bromelia pinguin* [18–22], as well as proteases from *B. plumieri* [23], *B. balansae* [24, 25], *B. hemisphaerica* [26], *B. hieronymi* [27–29], *B. fastuosa* [30], *B. antiacantha* [31], and *Pseudananas macrodontes* [32, 33].

P. macrodontes (Morr.) Harms (= *Pseudananas sagenarius* (Arruda) Camargo), is a terrestrial, stoloniferous plant (folk name "ihvirá"), whose infrutescences are smaller than those of *A. comosus*. Two cysteine endopeptidases have been isolated and biochemical characterized from their fruits: macrodontain I [32] and macrodontain II [33]. On the other hand, a partially purified extract of *P. macrodontes* fruits (PPE_{Pm}) has shown to possess anti-inflammatory activity [34].

The goal of this research is to advance in the knowledge of the proteases obtained from *P. macrodontes* through full sequencing of macrodontain I by Edman's degradation and its comparison with similar peptidases from members of the Bromeliaceae family. On the other hand, tests were carried out in order to determine if the anti-inflammatory activity of *P. macrodontes* extracts is related with its proteolytic activity. The presence of bioactivity in the plant extracts is of great interest, as could provide a source of biologically active compounds with therapeutic potential for humans.

Materials and Methods

Chemicals

Casein (from bovine milk), bromelain (B4882), chymomotrypsin, cysteine, pepsin, and Tris were obtained from Sigma-Aldrich, Inc. (St. Louis, MO, USA), endopeptidases Asp-N and Lys-C were obtained from Boehringer Ingelheim GmbH Corporate (Ingelheim, Germany), trypsin was obtained from Worthington Biochemical Corp. (Lakewood, NJ, USA), RP-C8 Acquapore HPLC columns were obtained from PerkinElmer (Waltham, MA, USA), and RP-C18 Supelco HPLC columns were obtained from Sigma-Aldrich (St. Louis, MO, USA). Ethylenediaminetetraacetic acid (EDTA) was purchased from Invitrogen (Carlsbad, CA, USA), sodium phosphate (98%) from Carlo Erba (Rodano, MI, Italy), ketamine chlorohydrate (50 mg/mL) from Holliday Scott (Beccar, BA, Argentina), xylazine chlorohydrate (10 mg/mL) from Richmond Laboratories Vet Pharma (Buenos Aires, Argentina), and dexamethasone sodium phosphate (4 mg/mL) from Laboratorios Klonal (Quilmes, BA, Argentina). Unless otherwise stated, all chemicals used in this study were of reagent grade.

Plant Material

Infrutescences of *Pseudananas macrodontes* were collected by Dr. Aníbal Amat in Santa Ana, province of Misiones, Argentina. A voucher specimen (No. 4544) was deposited at the University of Misiones, Pharmacy Department Herbarium, Argentina. After collection, fruits were washed with water, dried, and stored at − 70 °C.

Determination of Structural Properties of Macrodontain I

Enzyme Purification

Unripe but fully developed infrutescences of *P. macrodontes* were used for the isolation of macrodontain I by treatment with sodium phosphate buffer containing EDTA and cysteine, followed by acetone fractionation and ion exchange chromatography, as indicated in a previous communication [32].

Enzyme Digestions of Macrodontain I

Macrodontain I (4 mg) was inhibited with 1% iodoacetic acid and then treated with a denaturing reducing mixture (40% guanidine hydrochloride and 10 mM dithiotreitol), and alkylated with 25 mM iodoacetic acid. The sample was then diluted 10 times and independently digested with trypsin (1:40 enzyme protein ratio, pH 8.2, 3 h), pepsin (1:30 enzyme protein ratio, pH 2.0, 10 min), and Asp-N (1:50 enzyme protein ratio, pH 7.5, 18 h).

CNBr Cleavage of Macrodontain I

Macrodontain I was inhibited, denatured, reduced, and alkylated as indicated above and digested with CNBr using a 100-fold molar excess of CNBr to methionine residues. Reaction was ending by dilution with one volume of water and lyophilized.

Chromatographic Separation of Peptides

Peptides obtained from CNBr cleavage and enzyme digestion were separated by RP-HPLC (System Gold, Beckman) on a RP-C8 Acquapore, Brownlee column (4.6 × 250 mm) or a RP-C18 Supelco column (4.6 × 150 mm). Two solvent systems were used. System I was made up by solvent A (TFA-water, 1:500) and solvent B (acetonitrile-TFA-water 350:1:150). System II was made up by solvent A (TFA-water, 1:500) and solvent B (acetonitrile-TFA-isopropyl alcohol, 20:1:5). Initially, a 60 min linear gradient (1% up to a maximum of 70% of solvent B) was used, followed by a 10 min linear gradient up to a maximum of 95% of solvent B. According to the column used, elution rate was 0.8–1.0 mL/min. Peptide detection was carried out at 220 and 280 nm. Eluates were lyophilized and stored being before sequenced.

Amino Acid Sequence Determination

Sequence analysis of individual peptides was performed by Edman's automated degradation using an Applied Biosystems model 476 protein sequencer equipped with an on-line phenylthiohydantoin amino acid analyzer. Lyophilized peptides (0.5 to 1 nmol) were redissolved in a TFA-acetonitrile (0.1–50%) mixture and adsorbed onto a PVDF membrane, and then the membrane was inserted in the sequencer reaction chamber [35].

Glycoprotein Detection Using Concanavalin A

The purified enzyme was submitted to 12% SDS-PAGE and then blotted onto a nitrocellulose sheet. The blot was incubated with concanavalin A followed by horseradish peroxidase. The bound concanavalin A-peroxidase complex was detected using the chromogenic peroxidase substrate 4-chloro-1-naphthol [36]. Trasferrin was used as positive control.

Sequence Comparison

The protein sequence of macrodontain I was compared with those proteases from Bromeliaceae family that have been sequenced up to date: ananain [16], fastuosain [30], fruit bromelain [37], papain [38], hieronymain [29], and stem bromelain [39], using the ClustalW2.1 network service [40].

Homology Modeling of Macrodontain I

A multiple alignment of sequences of the plant cysteine proteases with known tertiary structure and macrodontain I was done using NCBI PDB-BLAST protein. Those sequences with major homology were selected as template for the homology modeling of macrodontain I (Table S1). In order to curate the template molecules for homology model of macrodontain I, these were prepared according to the following process: bond order assignment, addition of hydrogens, creation of disulfide bonds, and H-bonds assignment. Then, the secondary structures (ssa) of 2bdz (mexicain from *Jacaratia mexicana*), 1cqd (cysteine protease from *Zingiber officinale*), and 1iwd (ervatamin B from *Ervatamia coronaria*) were aligned by Prime software; from these, the secondary structure of macrodontain I was assigned (Table S2) to finally calculate its tertiary structure.

Determination of Anti-inflammatory Action of Cysteine Proteases of Pseudananas Macrodontes Fruits

Preparation and Characterization of PPE_{Pm}

Partially purificated extract (PPE_{Pm}) was obtained from infrutescences according to Errasti et al. [34]. Proteolytic activity was measured by caseinolytic activity assay (1% casein, 0.1 M Tris-HCl buffer, pH 7.5, 37 °C, 2 min), and arbitrary caseinolytic units (CU) were used to express proteolytic activity [32]. Protein content was measured by Bradford's method [41].

Preparation and Characterization of Inhibited PPE_{Pm} (PPE_{Pm}/E-64)

Cysteine proteases of PPE_{Pm} (20 mg/mL) were inhibited by incubation with E-64 (40 μM) during 30 min at 37 °C. Total inhibition of PPE_{Pm} was checked by the measurement of the caseinolytic activity as described above.

Matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS) was used for the characterization molecular of inhibited PPE_{Pm}. For MALDI-TOF target preparation, sample inhibited by E64 was diluted (1:1) in sinapinic acid matrix [10 mg/mL in acetonitrile: H_2O:TFA (500:499:1) solution], and 1 μL of mixture was spotted onto Precision AximaTM 384 plate using dried droplet method. Profile analyses were performed on an Axima Performance ID plus MALDI TOF/TOF spectrometer with Launchpad 2.9 (Shimadzu Biotech) data acquisition software. Mass spectra were acquired using the following settings: 600–5000 Da range, linear positive mode, ion source 20 kV, Einzel Lens 6 kV, pulsed ion extraction of 115 ns, gating start 12.67, and gating end 12. All spectra were obtained randomly over the spot surface manually. Mass calibration was performed externally using the ProteoMassTM Peptide and Protein MALDI-MS Calibration Kit (Sigma-Aldrich).

Anti-inflammatory Activity Assay

Comparison of anti-inflammatory activity of PPE_{Pm} and PPE_{Pm}/E-64 was performed by cotton pellet-induced granuloma test in rats following the method of Meier et al. [42] with some modifications [34]. Bromelain (Bro) and bromelain inhibited with E-64 (Bro/E-64) were tested for comparative purposes. Female Wistar albino rats weighing 115–150 g were used in the experiments. Animals were housed and cared for in the Animal Resource Facilities (Faculty of

Chemistry, Biochemistry and Pharmacy, National University of San Luis). The experimental protocols were approved by the Laboratory Animal Care and Use Institutional Committee in compliance with Argentine official resolutions for animal care guidelines (ANMAT No. 6344/96). Animals were randomly assigned to different groups ($n = 6$), provided with standard rodent chow diet and maintained at a constant temperature of 24 ± 1 °C and humidity of 55 ± 5% with 12 h light/dark cycle [34]. PPE_{Pm} dose was prepared by dissolving the lyophilized powder in sterile water, while bromelain dose was prepared by dissolving the commercial material in sterile 0.1 M sodium phosphate buffer (pH 6.0). PPE_{Pm}/E-64 and Bro/E-64 were prepared in the same way but followed by inhibition with E-64. As negative control, 0.1 M sodium phosphate buffer (pH 6.0) was used. Doses (0.5 mL) were administered intraperitoneally. The anti-inflammatory effect was assessed by determining the percentage inhibition of granuloma formation for each group in comparison with the control group as follows: $(1 - mt/mc) \times 100$, where mc and mt are the average weight of the granuloma of the control and treated groups, respectively. Similarly, percentages of weight reduction for thymus and spleens were calculated.

Statistical Analysis

GraphPad Prism software version 5.0 was used for statistical analysis. Data obtained are presented as mean ± SEM (standard error of the mean). Raw data were analyzed with one-way ANOVA (analysis of variance) followed by Tukey Multiple Comparison test. A probability of $p < 0.05$ was considered significant.

Results and Discussion

Structural Characterization of Macrodontain I

Because macrodontain I is an endopeptidase, denaturing and reduction of the protein are accompanied by autodigestion. To avoid this unwanted effect, the endopeptidase was initially inhibited with iodoacetic acid before reduction.

The proposed macrodontain I amino acid sequence was determined by overlapping the peptide sequences provided by the CNBr, trypsin, pepsin, and Asp-N digests (Fig. 1). As in macrodontain I sequence, three methionine residues were detected (Met121, Met122, and Met205), four CNBr peptides would be expected, but as two Met residues are occupying contiguous positions, the second peptide is non-existent and so only three peptides (B1, B2, and B3) were produced.

The primary structure of macrodontain I is composed of 213 amino acids with a calculated molecular weight of 23,486.18 Da, in close agreement with previous mass spectrometry data [32]. This sequence was deposited in UniProt (http://www.uniprot.org/uniprot) under the P83443 code.

The predicted molecular model of macrodondatin I (Fig. 2) establishes that the enzyme belongs to the α + β class of proteins, with two disulfide bridges (Cys23-Cys63 and Cys57-Cys96) in the α domain, while the β domain is stabilized by another disulfide bridge (Cys153-Cys201). The enzyme structure shows the typical papain-like fold composed of two domains separated by a groove containing the active site. A central groove divides the peptidase into two domains, with the active site located towards the bottom of this groove. The enzyme is

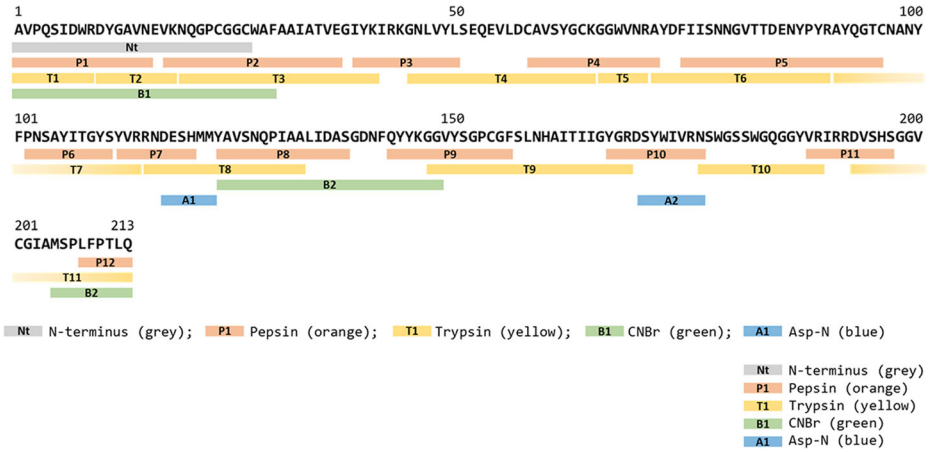

Fig. 1 Proposed amino acid sequence of macrodontain I. P1–P12 pepsin peptides, T1–T13 trypsin peptides, A1–A2 Asp-N peptides, B1, B3, and B4 CNBr peptides

fully inhibited by E64 (general cysteine protease inhibitor) and hydrolyses the synthetic peptide substrate Bz-Phe-Val-Arg-pNA (K_m 13.4 ± 0.8 μM), but not able to hydrolyze Bz-Arg-Arg-pNA substrate [33].

Sequence Comparison

Figure 3 shows the alignment of the primary structure of macrodontain I with those of all cysteine peptidases from species belonging to the family Bromeliaceae sequenced up to date and with papain the "prototype protease" from the subfamily C1A. The highest identity degree

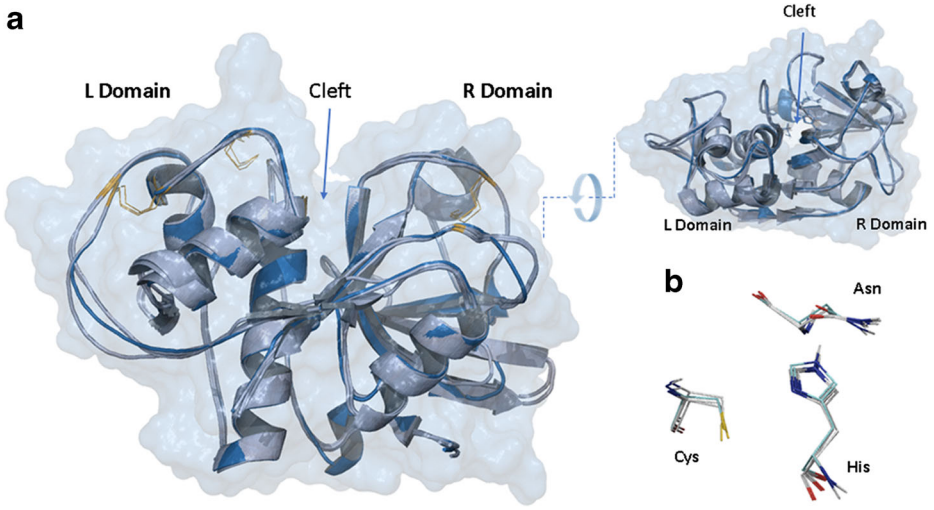

Fig. 2 Tertiary structure of macrodontain I predicted by molecular modeling. **a** Overlapped structure of macrodontain I with mexicain, cysteine protease from *Zingiber officinale*, and ervatamin is shown in cartoon style. R-L domains are indicated and disulfide bridges are represented with yellow sticks. **b** Overlapped stick structure of catalytic site residues of macrodontain I with mexicain, cysteine protease from *Zingiber officinale*, and ervatamin B

NAME | UNIPROT id	IDENTITY %
macrodontain_I|P83443	100.00
fruit_bromelain|O23791	87.74
hieronymain|D2U6V5	78.30
fastuosain|A1E254	73.21
ananain|P80884	72.99
stem_bromelain|P14518	67.62
papain|P00784	44.29

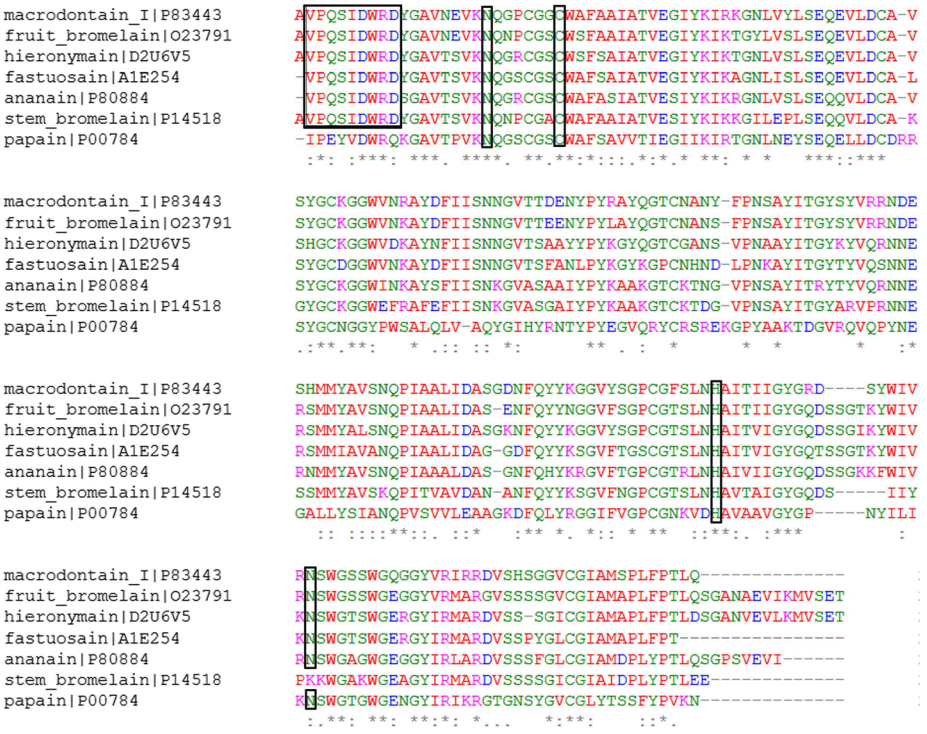

Fig. 3 Multiple sequence alignment by CLUSTAL-W of macrodontain I with proteases from the Bromeliaceae family (fruit bromelain, hieronymain, fastuosain, ananin, stem bromelain) and papain. Dashes represent gaps introduced to maximize alignment. Asterisk residues in the column are identical in all sequences in the alignment. Colon conserved substitutions. Dot residues in the column are semi-conserved substitutions. Box show the nonapeptid conserved in proteases from Bromeliaceae family and residues of the active site

(87.74%) was obtained against fruit bromelain [37], followed by hieronymain (78.3%) [29], fastuosain (73.21%) [30], ananain (72.99%) [16], and stem bromelain (67.62%) [39]. As expected, the macrondontain I sequence showed a lesser identity degree with papain, protease from Caricaceae family [38], than with proteases from Bromeliaceae family. A high conservation degree was observed particularly for those amino acid residues which are essential for catalytic activity: Cys26 and His159, which constitute the catalytic dyad in all cysteine peptidases, as well as active site residue Gln20 and Asn176, characteristic of Clan CA [43]. On the other hand, the residues Phe141, Trp178, and Trp182, involved in the hydrophobic pocket atf the catalytic site, are also conserved in macrodontain I. In addition to Cys26 residue of the active site, another six Cys residues are present in macrodontain I as part of three disulfide bridges, as mentioned. On the basis of the above information, macrodontain I should be included in the cysteine peptidase subfamily C1A.

A remarkable fact is that all Bromeliaceae peptidase sequences known up to date show two Met contiguous residues in the same positions (Met121 and Met122). Nevertheless, in papain (the peptidase type of the cysteine peptidase subfamily C1A), both Met residues are replaced by Leu residues. When sequences from the Bromeliaceae peptidases are compared, 119 amino acids (55.9%) are identical, most of them grouped in 22 motifs (VPQSIDWRD, GAV, VKNQ, CG, CWAF, IYKI, LSEQ, VLDCA, YGC, GGW, PY, QPI, DA, FQ, CG, LNHA, IGYG, WG, WG, GY, CG, and PT). The highest identity in proteasses from Bromeliaceae family is found at the N-terminal region (notably the nonapeptid VPQSIDWRD). This conserved sequence could be valuable for design of DNA primers.

Physicochemical Properties

Theoretical pI value of macrodontain I is 6.99, and the molar extinction coefficient is 61,685 M^{-1} cm^{-1}, at 280 nm measured in water, assuming all pairs of Cys residues form cysteinyl bridges (ExPASy ProtParam tool, http://ca.expasy.org/tools/protparam.html). Table 1 shows various physical and chemical parameters calculated for macrodontain I compared with those corresponding to cysteine proteases from family Bromeliacae sequenced so far. Molecular masses are as closely as expected (ranging from 22.8 to 24.9 kDa), but molar extinction coefficients showed a higher variation, evidencing a considerable difference in content of aromatic amino acids. Notably, a wide range of pI values are exhibited in the compared proteases (from 5.01 to 9.47). Moreover, it is noteworthy that macrodontain I is not a glycosilated peptidase, like ananain [15].

Anti-Inflammatory Action of Cysteine Proteases

In an early report, the anti-inflammatory effect of a partially purified extract obtained from *P. macrodontes* fruits (PPE_{Pm}) was detected in acute and chronic animal models [34]. In this paper, in order to know if cysteine proteases of *P. macrodontes* are involved in the anti-inflammatory action of PPE_{Pm}, the anti-inflammatory effects of PPE_{Pm} and PPE_{Pm} inhibited with E-64 (PPE_{Pm}/E-64) were determined. Mass spectrometry analysis was used as a approach for the characterization of PPE_{Pm}/E-64 to identify the peptidase-inhibitor complex. The mass spectrum (Fig. 4) shows several mass signals in agreement to cysteine proteases, detecting a main peak corresponding to the mass of macrodontain I/E64 (23,816 Da).

Table 1 Physico-chemical characteristics. Macrodontain I is compared with the sequenced peptidases from Bromeliaceae family

Endopeptidase	Mr	pI	ε (M^{-1} cm^{-1})	Glycoprotein
Macrodontain I	23,486.18	6.99	61,685	No
Ananain	23,411.59	9.47	52,745	No
Hieronymain	24,773.00	8.60	58,705	–
Fastuosain	23,380.31	7.71	55,725	–
Fruit bromelain	24,876.70	5.01	58,705	Yes
Stem bromelain	22,830.93	8.60	48,735	Yes

Mr relative molecular mass, *pI* isoelectric point, ε molar extinction coefficient

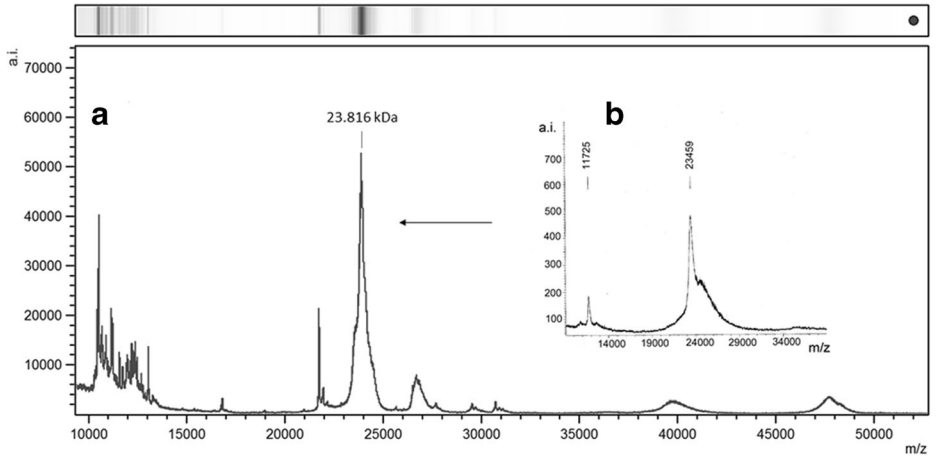

Fig. 4 **a** MALDI-TOF mass spectra of PPE_{Pm} inhibited by E64. **b** MALDI-TOF mass spectra of macrodontain I

For comparative purposes, the anti-inflammatory action of bromelain and bromelain inhibited with E-64 (Bro/E-64) was measured. Assayed doses (Table 2) were chosen according to the effective doses previously determined [34].

The adversed effects of dexamethasone like impairment of spleen and thymus weight as well as loss of body mass were measured. The percent inhibition of granuloma formation (anti-inflammatory action), as well as the percentage in weight reduction of thymus and spleen, is shown in Table 3. Both PPE_{Pm} and Bro had significant percentage inhibition of granuloma formation ($p < 0.05$) unlike those of PPE_{Pm}/E-64 and Bro/E-64. Neither the weight of spleens nor the body mass (data not showed) were significantly affected in animals treated with PPE_{Pm} and bromelain. However, a significant percentage reduction of thymus in animals treated with proteolytic extract was observed, that it was not detected in inhibited proteolytic extracts. Like in previous reports [34, 44], thymic atrophy caused by proteolytic extracts was much lower (about 25%) than that inflicted by dexamethasone (86%).

Taking into account these results and since E-64 is an irreversible inhibitor of cysteine proteases, anti-inflammatory action and the slight thymic atrophy could be attributed to cysteine proteases present in PPE_{Pm} as well as Bro (Table 3).

The knowledge that cysteine proteases of *P. macrodontes* fruits are involved in the anti-inflammatory effect of PPE_{Pm} allows us to direct future trials to elucidate the role that macrodontain I and macrodontain II have on inflammation as well as go deeper into the structure-function relation of cysteine plant proteases.

Table 2 Assayed doses of activated/inhibited extracts on cotton pellet-induced granuloma

Sample	Total mass (mg/kg body weight)	Protein (mg/kg body weight)	Proteolytic activity (CU/kg body weight)
PPE_{Pm}	90	2.2 ± 0.2	11 ± 1
PPE_{Pm}/E-64	90	2.2 ± 0.2	0.2 ± 0.1
Bro	50	10 ± 1	35 ± 3
Bro/E-64	50	10 ± 1	2 ± 3

Table 3 Effects of activated/inhibited extracts on cotton pellet-induced granuloma formation and weights of thymus and spleen in rats

	Granuloma		Thymus		Spleen	
	Weight (g)	Inhibition (%)	Weight (g)	Reduction (%)	Weight (g)	Reduction (%)
Control	0.50 ± 0.03		0.35 ± 0.02		0.68 ± 0.03	
PPE_{Pm}	0.35 ± 0.02	30*	0.26 ± 0.02	26*	0.9 ± 0.1	− 32
PPE_{Pm}/E-64	0.423 ± 0.006	15	0.33 ± 0.03	6	0.62 ± 0.07	9
Bro	0.37 ± 0.01	26*	0.27 ± 0.01	23*	0.9 ± 0.1	− 32
Bro/E-64	0.54 ± 0.05	− 8	0.39 ± 0.02	− 11	0.70 ± 0.08	− 3
E-64	0.50 ± 0.03	0	0.338 ± 0.007	3	0.7 ± 0.1	− 3
Dex[a]	0.232 ± 0.005	54***	0.048 ± 0.003	86***	0.33 ± 0.01	51**

[a] Dexamethasone (3 mg/kg body weight)

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ (Tukey's test)

Conclusions

Macrodontain I, one of the main peptidases of *P. macrodontes* fruits belonging to the cysteine peptidase subfamily C1A, was sequenced and compared with the others cysteine peptidases from Bromeliaceae. Notably, the nonapeptid VPQSIDWRD located in the N-terminal region is present in all of peptidases from Bromeliaceae family. Macrodontain I is a non-glycosilated peptidase composed by 213 amino acids with a calculated molecular weight of 23,486.18 Da, pI value 6.99, and molar extinction coefficient at 280 nm 61,685 M^{-1} cm^{-1}.

The cysteine peptidases of *P. macrodontes* are involved in the anti-inflammatory activity; this finding was also demonstrated in the case of bromelain.

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Compliance with Ethical Standards

Conflict of Interest The authors declare no conflict of interests.

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