Purification and characterization of a cysteine-rich secretory protein from Philodryas patagoniensis snake venom

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mammalian skeletal muscle of a CRiSP from the venom of

a force transductor (Ampère, Brazil) connected to a recording system (ECB, Brazil).

2.10. Statistical analysis

Where appropriate, the results were expressed as mean ± standard deviation (SD). Differences between groups were compared using one-way analysis of variance (ANOVA) followed by Tukey's test. Statistical analyses were performed using the software InfoStat/Professional, version 1.1. A value of $p < 0.05$ indicated statistical significance.

3. Purifi

protein yielded a molecular mass of 24,858.6 Da (Fig. 1-C). The peaks of 12,434.9 and 12,642.6 Da correspond to doubly-charged (z = 2) cationic forms.

The NH_2-terminal 14-amino acid sequence VDFDSESPRRPEIQ- (Uni-

separated protein bands were excised, in-gel digested with trypsin and the resulting peptides were analyzed by MALDI-TOF peptide mass fingerprinting followed by MALDI-TOF/TOF. The MALDI-TOF mass spectrum of the digested protein is shown in Fig. 3. The MS/MS spectrum of the fragmented singly-charged peptide ion (m/z = 1511.806) was matched by MASCOT to an internal sequence within the PR-1 (pathogenesis-related proteins of group 1) domain, MEWYAEAAANAER, from CRiSP-PHI1 and CRiSP-PHI2 of Philodryas olfersii (Ching et al., 2006; Fry et al., 2006). All of these results confirmed that a CRiSP from P. patagoniensis snake venom had been purified.

3.2. Patagonin activities

The purified protein, up to a final concentration of 400 μg/mL, hydrolyzed neither azocasein nor fibrinogen. When incubated with azocoll, patagonin (554 μg/mL, final concentration) did not degrade this substrate. It did not induce edema or hemorrhage, even at a dose of 20 μg. When added to washed human platelet suspensions or PRP, patagonin at concentrations up to 100 nM (final concentration)