Research paper

Alsophinase, a new P-III metalloproteinase with α-fibrinogenolytic and hemorrhagic activity from the venom of the rear-fanged Puerto Rican Racer *Alsophis portoricensis* (Serpentes: Dipsadidae)

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a r t i c l e i n f o

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Metalloproteinases from snake venoms are often multi-domain enzymes involved in degradation of a variety of structural proteins. Hemorrhage and tissue necrosis are common manifestations of viperid envenomations in humans, largely due to the actions of prominent metalloproteinases, and envenomation by rear-fanged snakes may also cause hemorrhage. We purified the major metalloproteinase in

resulting in often grave hemorrhage and tissue damage [9,10].

2.6. Fibrinogen digest

Fibrinogenase activity was determined using 1.5 μg of purified alsophinase incubated with human fibrinogen (final fibrinogen concentration of 1.0 mg/mL) in a total volume of 200 μL. 20 μL aliquots were removed at 0, 1, 5, 10, 30, and 60 min, combined with 20 μ

2.11. Local and systemic damage

additional peaks, was also observed after 4 or 24 h incubation (data not shown). The fractions comprising these peaks (1 h incubation) were SpeedVac dried and analyzed via MALDI-TOF MS. The observed molecular masses, the corrected expected masses, and the corresponding sequences of the fragments (as well as the intact peptide) are given in Table 1. The most labile bond was Ala14—Leu15, while the Tyr16—Leu17 bond was hydrolyzed at a much lower frequency under these assay conditions.

3.5. Inhibition assays

When alsophinase was incubated with the metalloproteinase inhibitor 1,10-phenanthroline, complete loss of proteinase activity was observed at concentrations as low as 50 μM 1,10-phenanthroline (Fig. 6A); the concentration at which 50% metalloproteinase activity was inhibited (IC_{50}) was approximately 15.2 μM. There appeared to be only minor inhibitory effects (at 1.0 and 5.0 mM) of the serine protease inhibitor AEBSF (Fig. 6B), as ~62% activity remained at both of these concentrations.

3.6. Hemorrhagic activity

Hemorrhagic activity of alsophinase was evaluated using the

injection also induced intramuscular hemorrhage, as evidenced by deep subcutaneous discoloration on the mouse intercostal muscles (not shown), and this extradermal effect may have contributed to the lower apparent dermal effects of the purified toxin.

3.7. Local and systemic damage

When crude *A. portoricensis* venom or purified alsophinase was injected intramuscularly into the gastrocnemius muscle of mice, hemorrhage and necrosis at the injection site were observed after 12 h for all dosages. Systemic damage was incurred in both the crude venom and alsophinase experimental groups, as evidenced by erythrocytic extravasation in the alveolar spaces and congestion of blood vessels in the lung tissue (Fig. 8A–C). Hematoxylin and eosin-stained muscle tissue samples reflected this observation; extravasation of erythrocytes and the presence of polymorphonuclear leukocyte infiltrate, which characterizes an inflammatory reaction, were seen in sectioned muscle (Fig. 8D–F).

4. Discussion

In this study, we purified and characterized the major metalloproteinase in *A. portoricensis* venom through size exclusion and ion exchange chromatography. Named alsophinase for *A. portoricensis* metalloproteinase, it is the fi

[48] F.S. Markland, Inventory of α- and β-fibrinogenases from snake venoms, Thromb. Haemost. 65 (1991) 1277–1285.
[49] A.S. Kamiguti, J.R. Slupsky, M. Zuzel, C.R. Hay, Properties of fibrinogen cleaved by jararhagin, a metalloproteinase from the venom of