
Since fibrinogen is a major risk factor following the onset of cardiovascular diseases, SVTLEs acting as *in vitro* defibrinogenating agents are potential candidates for the development of cardiovascular drugs and for treatment of hyperfibrinogenemia-associated disorders [7–9]. In addition, several SVTLEs currently or potentially find application as anticoagulant agents for the prevention and treatment of a wide range of thrombotic disorders, as a diagnostic reagent for the detection of fibrinogen levels in heparinized blood samples, and in coagulation studies [14].

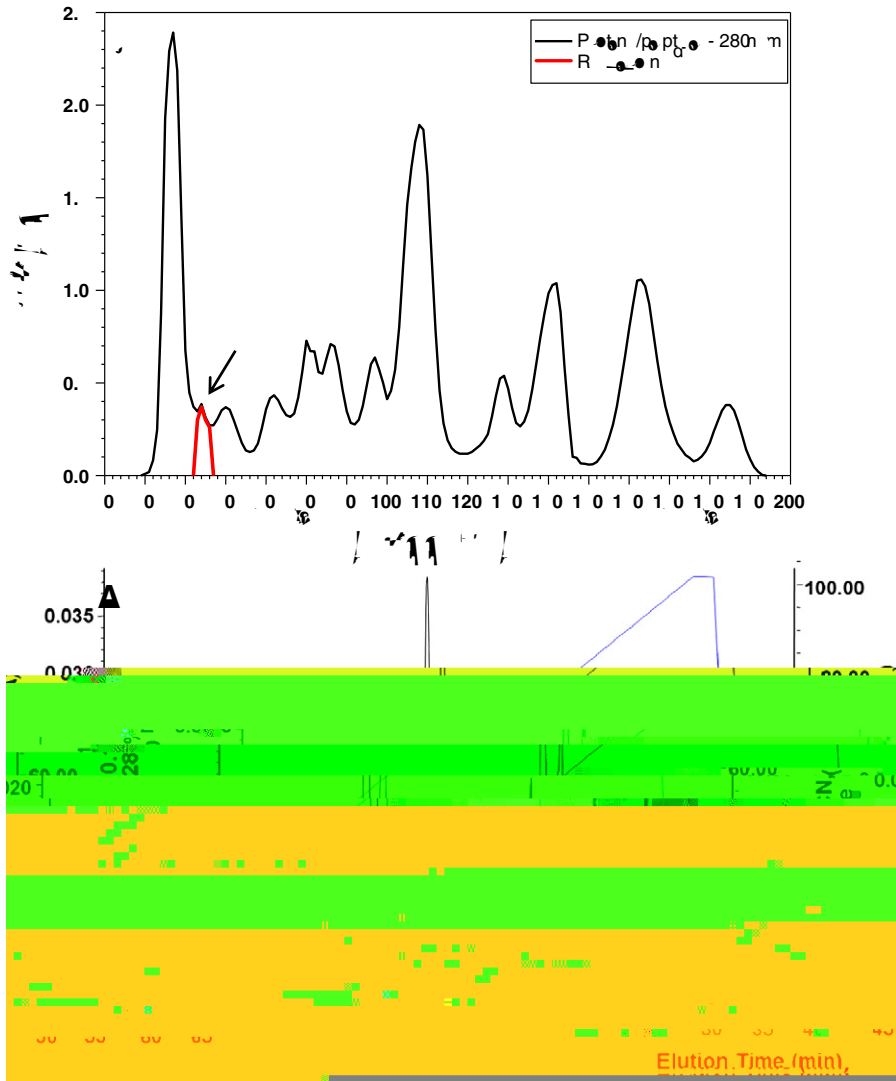
Most thrombin-like enzymes have been isolated, purified and char-

2.6. A fibrinogen clotting time

The fibrinogen clotting time was determined using a BBL-

2.12. Enzyme Inhibition

Inhibition of enzyme activity toward BzPheValArg-pNA or a native substrate (fibrinogen) was assayed as previously described after pre-incubation of 50 nmol of enzyme in 0.1 M HEPES buffer, containing 100 mM NaCl, pH 8.0 for 30 min at 37 °C containing one of the following inhibitors (final concentration): benzamidine-HCl (0.5–5 mM), aprotinin (100 µM), dithiothreitol (5–10 mM), diNa-EDTA (5–10 mM), heparin (100 IU/ml), soybean trypsin inhibitor (100–150 µg), α_2 -macroglobulin (100 µg), Antithrombin-III (100 µg), TPCK (100



	1	2	3	4	5	6	7
200 Da →							
116.3 Da ↕							
97.4 Da ↕							
66.3 Da →							
55.4 Da →							
36.5 Da →							
31.0 Da →							
21.5 Da →							
14.4 Da →							
6.0 Da →							
3.5 Da →							
2.5 Da →							

α_2 -macroglobulin and antithrombin-III showed a significantly higher inhibition ($p < 0.05$) of amidolytic (Fig. 6B) and fibrinogenolytic activity by partially deglycosylated Russelobin as compared to the native enzyme (data not shown).

3.13. Insulin B-chain cleavage by Russelobin and comparison with several representative snake venom proteases is depicted in Table 6.

Insulin B-chain cleavage by Russelobin and comparison with several representative snake venom proteases is depicted in Table 6.

4. D

Snake venoms consist of a myriad of biologically active proteins, and several, including thrombin-like SVSPs, have been developed either as potential drugs for the treatment of cardiovascular disorders or as diagnostic reagents [see 8,9 for recent reviews]. One of the most notable examples of this class of proteinase is Ancrod (Viprinex), a serine proteinase originally isolated from the venom of *Crotalus* (formerly

serine proteases. Russelobin catalyzed slow preferential hydrolysis at Val15 and Leu16 and it shows several additional minor cleavage sites at residues which are mostly not shown by other venom proteases.

5. C

Biological and biochemical characterization, N-terminal sequenc-

- serine proteinase, is a potent, specific fibrinogenolytic agent, *J. Thromb. Haemost.* 6 (2008) 1363–1372.
- [41] Y. Komori, T. Nikai, Chemistry and biochemistry of kallikrein-like enzyme from snake venoms, *J. Toxicol. Toxin Rev.* 17 (1998) 261–277.
- [42] J.W. Lee, J.H. Seu, I.K. Rhee, I. Jin, Y. Kawamura, W. Park, Purification and characterization of brevinase, a heterogenous two-chain fibrinolytic enzyme from the venom of Korean snake, *A₁ L₁ ... L₁ fi₁ ... L₁ L₁*. *Biochem. Biophys. Res. Commun.* 260 (1999) 665–670.
- [43] R.A.S. Ariens, Elevated fibrinogen causes thrombosis, *Blood* 117 (2011) 4687–4688.
- [44] A. Sahni, P.J. Simpson-Haidaris, S.K. Sahni, G.G. Vaday, C.W. Francis, Fibrinogen synthesized by cancer cells augments the proliferative effect of fibroblast growth factor-2 (FGF-2), *J. Thromb. Haemost.* 6 (2007) 176–183.
- [45] J. deO. Costa, K.C. Fonseca, C.C. Marnede, M.E. Beletti, N.A. Santos-Filho, A.M. Soares, E.C. Arantes, S.N. Hirayama, H.S. Selistre-de-Araújo, F. Fonseca, F. Henrique-Silva, N. Penha-Silva, F. de Oliveira, Bhalternin: functional and structural characterization of a new thrombin-like enzyme from *B₁ L₁ * L₁ ... L₁* snake venom, *Toxicon* 55 (2010) 1365–1377.
- [46] R.M.R. Torrent, B. Bongiovanni, L.C. Leiva, A.M.E. Duffard, J.P. Rodriguez, O.C.A. Pérez, R. Duffard, Neurotoxicological effects of a thrombin-like enzyme isolated from *C₁ L₁ L₁ L₁ ... fi₁ L₁* venom (preliminary study), *Toxicon* 50 (2007) 144–152.
- [47] C.A. Ariaratnam, M.H. Sheriff, C. Arambepola, R.D. Theakston, D.A. Warrell, Syndromic approach to treatment of snake bite in Sri Lanka based on results of a prospective national hospital-based survey of patients envenomed by identified snakes, *Am. J. Trop. Med. Hyg.* 81 (2009) 725–731.