Since fibrinogen is a major risk factor following the onset of cardiovascular diseases, SVTLEs acting as 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].

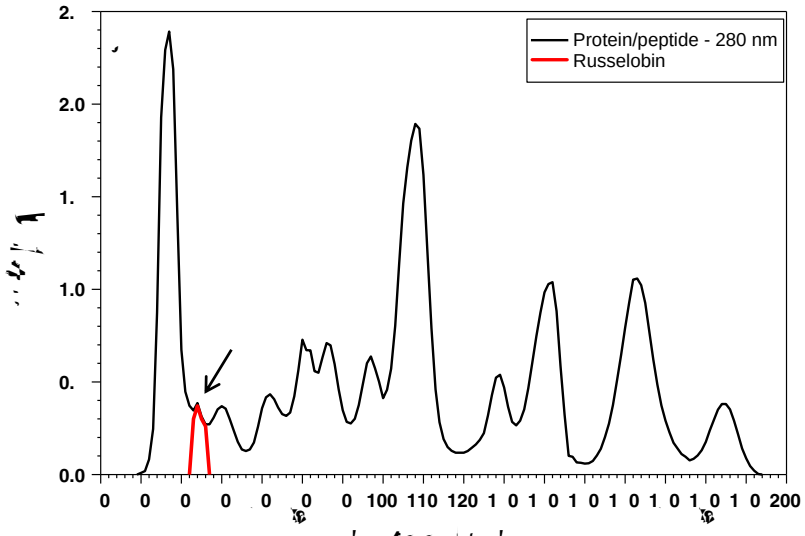
Most thrombin-like enzymes have been isolated, purified and char-

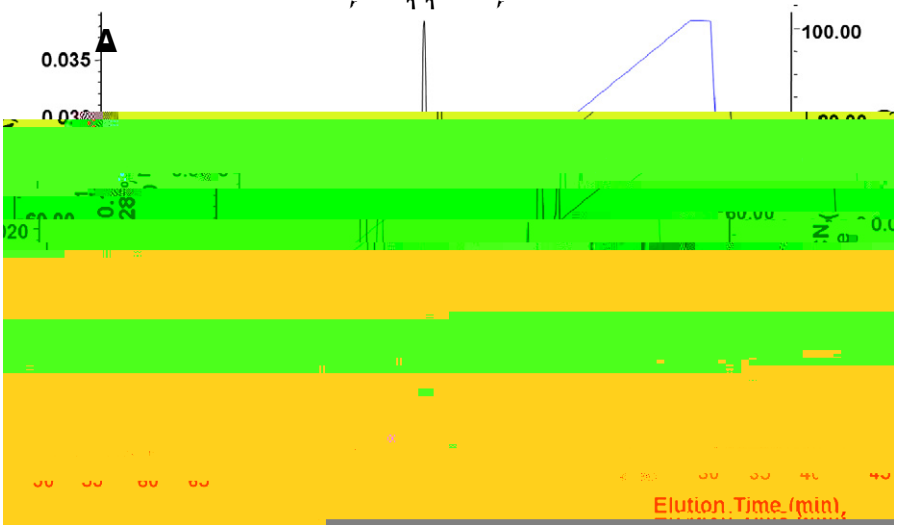
2.6. A [illegible]

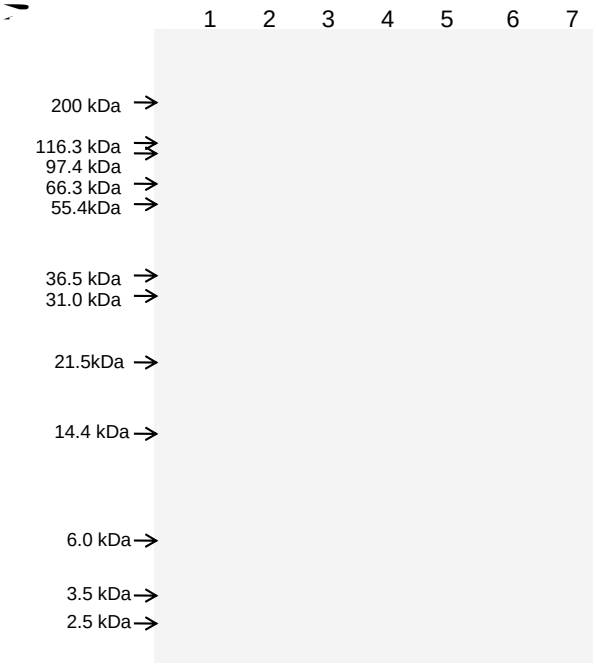
The fibrinogen clotting time was determined using a BBL-

2.12. E [illegible]

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


Protein/peptide - 280 nm
Russelobin
2.
2.0
1.
1.0
0.
0.0
0 0 0 0 0 0 0 100 110 120 1 0 1 0 1 0 1 0 1 0 1 0 1 0 200


A
0.035
100.00
Elution Time (min),


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

Fibrinopeptide release from fibrinogen after incubation with Russelobin is depicted in Fig. 5. The release of fibrinopeptides A and B was confirmed by MALDI-TOF-MS analysis of the RP-HPLC peaks of fibrinogen degradation products. The peptide peaks eluting at 16.73 min and 17.33 min showed masses of 1537.229 [M + H] and 1553.159 [M + H], respectively, corresponding to the masses of FPA and FPB. Russelobin at 40 nM did not release detectable quantities of either fibrinopeptide A or B; however, after increase in the enzyme concentration to 80 nM, preferential release of FPA was observed. The release of FPB was observed at a much slower rate, approaching the maximum value only after 8 h of incubation at room temperature (data not shown). Human thrombin, on the other hand, at a concentration of 5.0×10^{-5} NIH U/ml, released almost equal amount of FPA (1.2 nmol) and FPB (1.1 nmol) after 8 h at room temperature (data not shown).

3.9. E ... FA FB fi ... ... t ...

Russelobin-mediated degradation of fi

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. [illegible] B-[illegible]

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 *C* (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, [illegible], Biochem. Biophys. Res. Commun. 260 (1999) 665–670.
[43] R.A.S. Ariëns, 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. Mamede, 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 [illegible] snake venom, Toxicon 55 (2010) 1365–1377.
[46] R.M.R. Torrent, B. Bongiovanni, L.C. Leiva, A.M.E. Duffard, J.P. Rodríguez, O.C.A. Pérez, R. Duffard, Neurotoxicological effects of a thrombin-like enzyme isolated from [illegible] 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.