Higa et al. BMC Proceedings 2014, 8(Suppl 4):P12
http://www.biomedcentral.com/1753-6561/8/S4/P12

BMC Proceedings

POSTER PRESENTATION Open Access

Degradation of Aα and Bβ chains from bovine fibrinogen by serine proteases of the Amazonian scorpion *Brotheas amazonicus*

Andre Higa[1*], Maria das Dores Noronha[2], Jorge Luis López-Lozano[2]

From 5th Congress of the Brazilian Biotechnology Society (SBBIOTEC)
Florianópolis, Brazil. 10-14 November 2013

Background

Proteolytic enzymes within venoms from different scorpion species belong to serine and metalloproteases class, and some isoforms of these enzymes show proteolytic activity over Aα and Bβ subunits of fibrinogen [1-3]. *Brotheas amazonicus* is a non-lethal amazonian scorpion, belonging to a different group from *Tityus* genus, and there are scarce studies about its venom in literature [4]. Enzymes acting over fibrinogen can lead to the creation of more efficient antithrombotic drugs, and the low lethality of *B. amazonicus* venom is a potencial factor for developing a better drug.

Material and methods

Venon was early incubated with metallo or serine proteases inhibitors (PMSF and EDTA), and proteolytic activity was evaluated by zymogram in SDS-PAGE using as substrate bovine fibrinogen. After electrophoresis, gel was rinsed with 2.5% Triton X-100. Gel was incubated in humid chamber for 24 hours at 37°C, in 0.1M Glycine pH 8.3 solution. Dye was performed using 0.02% Comassie Blue R-250 solution. Proteolytic activity of *B. amazonicus* venom over bovine fibrinogen was tested, by mixing 200 μL of fibrinogen solution (2μg/μL) in 0.01M PBS pH 7.4 with 50 μg of *B. amazonicus* venom, for 12 hours at 37°C. After this process, no clots were observed, so 5μg of *Bothrops atrox* venom [5] was incorporated to the system, but also no clotting was observed, suggesting *B. amazonicus* venom had activity over fibrinogen, but with no clotting formation. Changes induced by *B. amazonicus* venom on bovine fibrinogen were evaluated by 12% SDS-PAGE electrophoresis stained with silver nitrate. Inhibition efficacy of fibrinogenolytic activity of *B. amazonicus* venom by anti-scorpionic serum was tested, by adding different concentrations of anti-venom in 200μL of bovine fibrinogen (2μg/μL) plus 5μg of *B. amazonicus* venom. This system was incubated for 24 hours at 37°C, and after this process clotting induction by 5μg of *B. atrox* venom was performed.

Results and conclusion

Proteolytic activity of *B. amazonicus* venom over bovine fibrinogen was only inhibited by PMSF - specific inhibitor for serine proteases. *B. amazonicus* venom degraded bovine fibrinogen without fibrin clots formation, confirmed by clots absence when *B. atrox* venom was incorporated to the system. In SDS-PAGE electrophoresis of degraded fibrinogen, it was possible to detect that *B. amazonicus* venom degraded Aα and Bβ subunits of fibrinogen, and anti-scorpionic serum specific for *Tityus* species shows great neutralizing efficacy when 1:1 proportion, suggesting that *B. amazonicus* toxins show similar antigenic properties of serine proteases from *Tityus* genus venom. Results suggest a serine protease with bovine fibrinogen affinity, able to degrade different regions from this molecule unlike thronbin and with a high similarity of proteases from *Tityus* sp. Such characteristics, plus the fact that this venom has a low toxicity, make these proteases inside *B. amazonicus* venom as candidates for antithrombotic drugs or even vaccines against scorpionic accidents.

Acknowledgements
Amazonas State Government, Brazilian Ministry of Science and Technology and National Council of Development in Research and Technology (CNPq)

Authors' details
[1]Universidade do Estado do Amazonas, Manaus, Amazonas, Brazil. [2]Fundação de Medicina Tropical Doutor Heitor Vieira Dourado, Manaus, Brazil.

[1]Universidade do Estado do Amazonas, Manaus, Amazonas, Brazil
Full list of author information is available at the end of the article

Published: 1 October 2014

References

1. Almeida FM, Pimenta AM, de Figueiredo SG, Santoro MM, Martin-Eauclaire MF, Diniz CR, de Lima ME: **Enzymes with gelatinolytic activity can be found in Tityusbahiensis and Tityusserrulatus venoms.** *Toxicon* 2002, **40(7)**:199-203.
2. Araújo TF, Higa AM, Noronha MDN, Martins MJP, Muniz EG, Medeiros BM, Aguiar NO, López-Lozano JL: **Proteolytic activity of amazonian scorpion Tityus metuendus venom (Scorpiones: Buthidae).** *J Venom Anim Toxins incl Trop Dis* 2007, **13(1)**:254.
3. Possani LD, Martin BN, Fletcher MD, Fletcher PL Jr: **Discharge effect on pancreatic exocrine secretion produced toxins purified from Tityus serrulatus scorpion venom.** *J Biomol Chem* 1991, **266**:3178-3185.
4. Lourenço WR, Adis J: **Amazonian Arachnida and Myriapoda.** 2007, **4(9)**:399-438.
5. López-Lozano JL, Valle de Sousa M, Ricart CAO, Chávez-Olortegui C, Flores-Sánchez E, Muniz EG, Bührnheim PF, Morhy L: **Ontogenetic variation of metalloproteinases and plasma coagulant activity in venoms of wild Bothrops atrox specimens from Amazonian rain forest".** *Toxicon* 2002, **40**:997-1006.

doi:10.1186/1753-6561-8-S4-P12
Cite this article as: Higa *et al.*: Degradation of Aα and Bβ chains from bovine fibrinogen by serine proteases of the Amazonian scorpion *Brotheas amazonicus*. *BMC Proceedings* 2014 **8**(Suppl 4):P12.