Venom proteomes of South and North American opisthoglyphous (Colubridae and Dipsadidae) snake species: A preliminary approach to understanding their biological roles

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and ecchymotic lesions on the bitten limb, often bearing a striking resemblance to the local signs and symptoms of Bothrops sp. envenomations (Kuch and Jesberger, 1993; Nishioka and Silveira, 1994; de Araujo and dos Santos, 1997; Ribeiro et al., 1999; de Medeiros et al., 2010).

Hypsiglena (family Dipsadidae) and Trimorphodon (family Colubridae) (

2.7. Mass spectrometry

Approximately 1 μg crude venoms in 50% ACN containing 0.1% TFA was spotted onto a MALDI sample holder, mixed with an equal volume of 10 mg/mL sinapinic acid in 50% ACN containing 0.1% TFA, and allowed to dry. Mass spectra were obtained using a Bruker Ultraflex II MALDI-TOF/TOF mass spectrometer (Proteomics and Metabolomics Facility, CSU, Fort Collins, CO, USA) in linear mode using a 25 kV accelerating voltage and calibrated with an external protein standard (5 proteins, 6–140 kDa). Putative protein families of common venom proteins known to occur in rear-fanged snake venoms (e.g.,

Chromatograms (Fig. 3) from the three Philodryas venoms and HttV were similar but greater variation was seen in TblV, in which three additional protein peaks were revealed. One of these corresponded to the PLA_2-active fraction of the venom, and the other two corresponded to proteins of ~9 and 18 kDa, both of which showed homology with 3FTx-Tri2 (Fry et al., 2008), a three-finger toxin from TblV (see below). Minor peaks corresponding to 6–20 kDa protein bands (data not shown) were also revealed in chromatograms from the three Philodryas venoms and HttV (Fig. 3), indicating that these peptides are expressed at much lower levels in these venoms.

3.4. Mass spectrometry

MALDI-TOF mass spectra of the five crude venoms revealed a diversity of proteins (Fig. 4), complementing 2D SDS-PAGE, and approximately 40 proteins with unique masses (difference of > 2%) were resolved; protein family identity was assigned based on characteristic masses (Supplementary Table 1). Three finger toxins (masses ~7.8–8.5 kDa) were present in the venoms of Poo, Htt and Tbl, but they were major components only in Tbl venom. Consistent with enzyme assays, proteins assigned to PLA_2 (masses ~13.8–14.2 kDa) were present only in Tbl venom, but proteins with similar masses (<13.5, > 14.5 kDa) were present in the other venoms. Proteins with masses of C-type lectins (masses ~15.3–16.2 kDa) were found in venoms of Pp, Poo and Tbl.

Peichoto et al., 2007; Weldon and Mackessy, 2010, 2012). These enzymes degrade basement membrane structure and weaken and disrupt the capillary wall, which leads to bleeding (Acosta et al., 2003) as well as inflammatory effects (

2006). Another thing worth noting is that snake venom matrix metalloproteinases (svMMPs), major components recently discovered in the venom of the dipsadid T. strigatus (Ching et al., 2012), do not seem to be abundant components in the venoms analyzed here, including those from species belonging to the same family of T. strigatus (Pp, Poo, Pb

Acosta, O., Leiva, L.C., Peichoto, M.E., Maruñak, S., Teibler, P., Rey, L., 2003. Hemorrhagic activity of the Duvernoy's gland secretion of the xenodontine colubrid Philodryas patagoniensis from the north-east region of Argentina. Toxicon 41, 1007–1012.

Antunes, T.C., Yamashita, K.M., Barbaro, K.C., Saiki, M., Santoro, M.L., 2010. Comparative analysis of newborn and adult Bothrops jararaca snake venoms. Toxicon 56, 1443–1458.

Assakura, M.T., Salomão, M.G., Puorto, G., Mandelbaum, F.R., 1992. Hemorrhagic, fibrinogenolytic and edema-forming activities of the venom of the colubrid snake Philodryas olfersii (green snake). Toxicon 30, 427–438.

Assakura, M.T., Reichl, A.P., Mandelbaum, F.R., 1994. Isolation and characterization of five fibrin(ogen)olytic enzymes from the venom of Philodryas olfersii (green snake). Toxicon 32, 819–831.

Blum, H., Beier, H., Gross, H.J., 1987. Improved silver staining of plant proteins, RNA and

Salomão, E.L., Di-Bernardo, M., 1995. Philodryas olfersii: uma cobra comum que mata: caso registrado na área da 8ª Delegacia Regional de Saúde. Arq. Soc. Bras. Zool./Sorocaba 14–16 (21).

Salomão, M.G., Albolea, A.B.P., Santos, S.M.A., 2003. Colubrid snakebite: a public health problem in Brazil. Herpetol. Rev. 34, 307–312.

Scott, D.L., Otwinowski, Z., Gelb, M.H., Sigler, P.B., 1990a. Crystal structure of bee-venom phospholipase A_2 in a complex with a transition-state analogue. Science 250, 1560–1566.

Scott, D.L., White, S.P., Otwinowski, Z., Yuan, W., Gelb, M.H., Sigler, P.B., 1990b. Interfacial catalysis: the mechanism of phospholipase A_2. Science 250, 1541–1546.

Vejayan, J., Yee, L.S., Ponnudurai, G., Ambu, S., Ibrahim, I., 2010. Protein profile analysis of Malaysian snake venoms by two-dimensional gel electrophoresis. J. Venomous Anim. Toxins Incl. Trop. Dis. 16, 623–630.

Vellard, J., 1955. Propriétés venimeuses de Tachymenis peruviana Wiegm. Folia Biol. Andina Pars II — Zool. 1, 1–14.

Verheji, H.M., Volwerk, J.J., Jasen, E.H.J.M., Puyk, W.C., Dijkstra, B.W., Drenth, J., Haas, G.H., 1980. Methylation of histidine-48 in pancreatic phospholipase A_2. Role of histidine and calcium ion in the catalytic mechanism. Biochemistry 19, 743–750.

Vest, D.K., 1988. Some effects and properties of Duvernoy's gland secretion from Hypsiglena torquata texana (Texas night snake). Toxicon 26, 417–419.

Vidal, N., 2002. Colubroid systematics: evidence for an early appearance of the venom apparatus followed by extensive evolutionary tinkering. J. Toxicol. Toxin Rev. 21, 21–41.