Chapter 9
Translationally Controlled Tumor Protein (TCTP/HRF) in Animal Venoms

Andrea Senff-Ribeiro

Abstract Proteins from TCTP/HRF family were identified as venom toxins of spiders from different genus. We have found a TCTP toxin in the venom gland of *Loxosceles intermedia*, a venomous spider very common in South Brazil. TCTP from *L. intermedia*, named LiTCTP, was cloned, produced in a heterologous prokaryotic system, and the recombinant toxin was biochemically characterized. Our results point that LiTCTP is involved in the inflammatory events of Loxocelism, the clinical signs triggered after *Loxosceles* sp. bite, which include intense inflammatory reaction at the bite site followed by local necrosis. TCTP toxins were also identified in spiders from different genus. There are very few articles about TCTP toxins in other venomous animals in the literature, although a NCBI database search on the protein sequences reveals TCTP on snake's venom glands transcriptomic and genomic studies. Studies on TCTP as a venom toxin are very few and its biological role as a venom component in prey capture is still unknown.

9.1 Introduction

Proteins from TCTP/HRF family have already been described in the gland secretion of arthropods, venom, and saliva (Gremski et al. 2014). TCTP/HRF activates multiple human cells including basophils, eosinophils, T cells, and B cells, which participate in the allergic response (MacDonald 2012). Therefore, extracellular functions of HRF/TCTP may exacerbate the allergic and inflammatory cascade observed in venomous accidents. These TCTP venom toxins were identified in venom glands of spiders from different genus (Sade et al. 2012; Kimura et al. 2012; Zobel-Thropp et al. 2014). We have studied a TCTP from *Loxosceles intermedia* venom. *Loxosceles* spiders are encountered in all continents and more than 100 different species have been reported. *Loxosceles* sp. spiders are venomous animals whose bites trigger a set of clinical signs called Loxocelism. Victims present

A. Senff-Ribeiro (✉)
Department of Cell Biology, Federal University of Paraná, Curitiba, Paraná, Brazil
e-mail: senffribeiro@gmail.com; senffribeiro@ufpr.br

A. Telerman, R. Amson (eds.), *TCTP/tpt1 - Remodeling Signaling from Stem Cell to Disease*, Results and Problems in Cell Differentiation 64,
DOI 10.1007/978-3-319-67591-6_9

intense inflammatory reaction followed by local necrosis with gravitational lesion spreading at the bite site and occasional systemic manifestations (Vetter 2008). The TCTP toxin from *L. intermedia* venom (LiTCTP) is involved in the inflammatory events of Loxoscelism. LiTCTP directly causes edema and increases vascular permeability in mice (Sade et al. 2012). In some cases, *Loxosceles* spider bites can cause hypersensitivity or even allergic reactions (Rattmann et al. 2008).

TCTP protein was also described in the venom of spider species: Gtx-TCTP was identified in *Grammostola rosea* tarantula venom gland (Kimura et al. 2012), SthTCTP in *Scytodes* spiders (Zobel-Thropp et al. 2014). But these toxins were not biochemically or biologically characterized.

Although research articles on TCTP from spiders are very few, we can find several protein sequences related to TCTP/HRF on NCBI database; there are also TCTP sequences described in snakes, but no related protein in scorpions.

9.2 *Loxosceles intermedia* TCTP

Loxosceles (brown spiders) is one of the medically important spider's genuses, which also include Lactrodectus (widow spiders), Phoneutria (armed spiders), and Hadronyche spp. (funnel-web spiders). Five species of Loxosceles are responsible for most cases of human envenomation (*L. rufescens, L. laeta, L. intermedia, L. gaucho*, and *L. reclusa*) (da Silva et al. 2004). The classical clinical symptoms of the dermonecrotic lesion caused by *Loxosceles* spider bites includes erythema, itching, and pain and are classified as cutaneous Loxoscelism (more than 70% of the cases) (Gremski et al. 2014). Accidents can also cause hypersensibility or even allergic reactions, symptoms which could be associated with histaminergic events such as an increase in vascular permeability and vasodilatation. Loxosceles venoms have a complex molecular composition, enriched with proteins that range from 3 to 40 kDa in molecular mass. The venom is composed of several different toxins and the mechanisms by which the venom exerts its effects are still under investigation, although studies have shown that venom components act synergistically (Gremski et al. 2014).

A TCTP/HRF protein was identified in the cDNA library of the *L. intermedia* venom gland. The complete cDNA sequence of LiTCTP comprises 536 bp and has an open reading frame that encodes a deduced 172-amino acid protein. The calculated molecular mass of the mature protein for LiTCTP was 22.3 kDa and the predicted pI 4.7 (Sade et al. 2012). We performed the cloning, heterologous expression, purification, and functional characterization on this novel member of the TCTP family from the *L. intermedia* venom gland (Sade et al. 2012). TCTP from *Loxosceles intermedia* (LiTCTP) was cloned and expressed as a heterologous protein in an *E. coli* expression system as a fusion protein with a 6× His-tag at the N-terminus. Purification of LiTCTP was performed by two steps chromatography, immobilized metal–ion affinity chromatography (Ni-NTA) agarose and ion exchange chromatography (DEAE-sepharose).

Transcriptome analysis of *L. intermedia* venom gland revealed that TCTP coding transcripts represent 0.4% of the encoded toxins (Gremski et al. 2010). LiTCTP recombinant protein and native *L. intermedia* venom toxins showed immunological cross-reactivity by immunoblot and ELISA assays (Sade et al. 2012). Recently, we showed there are also TCTP-related proteins in the venoms of *L. laeta* and *L. gaucho* by the detection of LiTCTP-related epitopes on toxins from these venoms (Buch et al. 2015).

LiTCTP is most homologous to TCTP from the ixodid ticks *Ixodes scapularis* and *Amblyomma americanum* (69% of sequence identity) and the tick *Dermacentor variabilis* (68% of identity). Phylogenetic analysis demonstrates that the *L. intermedia* TCTP protein is closely related to histamine releasing factors of ixodid ticks.

Most of the symptoms observed during Loxoscelism can be triggered by the phospholipase-D toxins, the most characterized and studied class of toxins from Loxosceles venoms. However, recombinant isoforms of phospholipase-D were not able to induce paw edemas of the same intensity as observed in whole venom tests. The edema at the bite site is a well-described symptom of cutaneous Loxoscelism (Ribeiro et al. 2007; Paludo et al. 2009). As TCTP is histamine releasing factor, we investigated the edematogenic effect of recombinant LiTCTP in a mouse model. LiTCTP induced subcutaneous paw edema in vivo in a time- and concentration-dependent manner. The LiTCTP edematogenic effect started rapidly (5 min) decreasing until a minimum thickness was reached after 240 min (Sade et al. 2012).

The effects of LiTCTP in vascular permeability were evaluated by observing vascular leakage of Evans Blue dye into the extravascular compartment of the skin in mice injected with the toxin (Sade et al. 2012). The dye leakage pattern varied between the vascular effect of the venom and the recombinant toxin, venom showed several extravasation points near the injection site, and LiTCTP presented a more diffuse profile. It has already been shown that *L. intermedia* venom can degranulate mast cells and release mediators such as histamine, which increase vascular permeability and induce vascular relaxation (Paludo et al. 2009). LiTCTP seems to be an earlier and quicker component of edema formation compared with the inflammatory response in mouse paws triggered by whole venom (Sade et al. 2012). In the context of Loxoscelism, extracellular functions of HRF/TCTP could exacerbate the inflammatory cascades and allergic response by the activation of immune cells involved in these process (including basophils, eosinophils, T cells, and B cells) contributing to the clinical signs observed following *Loxosceles* spider's bites.

The TCTP biological role as a Loxosceles venom component in prey capture is still unknown. In the case of the *Loxosceles* spiders, TCTP and other constituents of whole venom are secreted via holocrine secretion by venom gland as revealed by ultrastructural studies of the venom gland (dos Santos et al. 2000). Maybe TCTP is present in the Loxosceles venom because it is a cellular component of venom gland cells, as other cytoplasmatic proteins which were also described in the transcriptome study (Gremski et al. 2010).

9.3 Other TCTPs Found in Animal Venoms

Other studies in spider toxinology also described TCTP toxins. A TCTP sequence was identified in the cDNAs encoding toxin-like peptides from the venom gland of the Chilean common tarantula *Grammostola rosea* (Kimura et al. 2012). This spider belongs to the Theraphosidae family of spiders, which are large and often hairy arachnids with a widespread distribution throughout the tropics and subtropics (Escoubas and Rash 2004). Unlike *Loxosceles*, most of these animals are not involved in venomous accidents with humans (Escoubas and Rash 2004). After ESTs techniques applied to the cDNA library, Gtx-TCTP was revealed in *Grammostola rosea* tarantula venom gland (Kimura et al. 2012). GTx-TCTP transcript was expressed in both the venom gland and the pereopodal muscle. Real-time PCR showed that GTx-TCTP transcript in the pereopodal muscle was one-13th of that in the venom gland. Authors assume that GTx-TCTP acts as both growth-related cytosolic protein and secretory protein and that further investigation is needed to elucidate the bifunctional features of GTx-TCTP (Kimura et al. 2012).

TCTP is among the proteins expressed in the venom gland of *Scytodes thoracica*. This genus has a worldwide distribution and contains nearly 200 species. In *Scytodes* transcriptome and proteomic study, three cDNAs were identified as SthTCTP (*S. thoracica* TCTP). The molecular mass of SthTCTP amino acid sequence is predicted to be 19.3 kDa with a pI of 4.63. Phylogenetic analysis and several significant hits ($e \leq 10^{-5}$) from NCBI support a close relationship between SthTCTP and LiTCTP (86% of identity). *Scytodes* is a close relative of sicariids, including *Loxosceles* and *Sicarius* spiders whose venoms are toxic to mammals (Vetter 2008).

These are the only data on spiders TCTP available in scientific literature, but a simple search on protein sequences related to TCTP/HRF on NCBI database finds 10 TCTP sequences from spiders: Scytodidae *(3)*, Theraphosidae *(3)*, Sicariidae *(2)*, Lycosidae *(1)*, Theridiidae *(1)* families. Figure 9.1 shows the alignment of TCTP sequences from spiders and human. There is a TCTP partial sequence from *Lycosa singoriensis* venom gland on the database, although this sequence is not specifically mentioned as a toxin-like or a cellular transcript in the related manuscript (Zhang et al. 2010). Concerning spiders from *Theridiidae* family, *Latrodectus hesperus* (western black widow), it is not clear if the partial sequence was identified in venom glands or other part of the spider. From *Theraphoside* family, besides the sequence from *Grammostola rosea*, there is also a partial TCTP sequence from the spider *Haplopelma schmidti* (*Selenocosmia huwena*) on the database but the results of the study are still unpublished. The database also points to 16 TCTPs sequences from mites and ticks, other arthropods.

There is no data on the literature about TCTP in other venomous animals, but a search on the protein sequences from NCBI database (http://www.ncbi.nlm.nih.gov/protein) reveals 10 TCTP sequences on snakes: from *Viperidae (5)*, *Elapidae (3)*, *Colubridae (1)*, and *Pythonidae (1)* families. Figure 9.2 shows the alignment of TCTP sequences from snakes. Venoms from *Viperidae* and *Elapidae* families

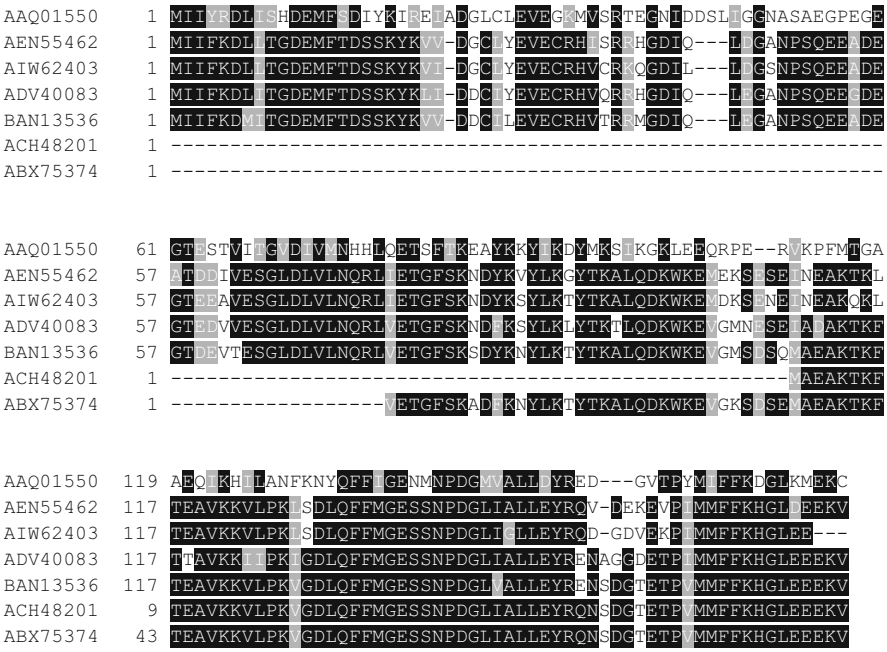

Fig. 9.1 Multiple alignment of TCTP protein sequences from different species of spiders compared to human and *Loxosceles intermedia* TCTP. Sequence alignment was performed using the Clustal Omega program and formatted with the BOXSHADE program (version 3.21). Fully conserved positions are shaded in *black* and conservative substitutions are in *gray*. GenBank sequences: *Homo sapiens* (AAQ01550.1); *Loxosceles intermedia* (AEN55462.1); *Scytodes thoracica* (AIW62403.1); *Grammostola rosea* (BAN13536.1); *Haplopelma schmidti* (ACH48201.1); *Latrodectus Hesperus* (ADV40083.1); *Lycosa singoriensis* (ABX75374.1)

present direct and negative impacts on human health. *Crotalus adamanteus* and *Crotalus horridus* (*Viperidae* family) TCTPs are on the database. *C. adamanteus,* the largest member of the genus, is a pit viper native to the southeastern United States whose TCTP was identified in its venom gland transcriptome. TCTP was also found in the venom gland transcriptome from a specific population of *C. horridus*, which presents potent and lethal venom and is found in northern Florida (USA). Interestingly this venom does not have the hemorrhagic effects typical of rattlesnake bites. *Micrurus fulvius* (eastern coral snake) and Ophiophagus are New World coral snakes (*Elapidae* family). TCTPs identified in the venom gland transcriptome from these venomous snakes are on the database. Although, these TCTP sequences which are in the database are not mentioned in the reference manuscripts (Rokyta et al. 2013; Rokyta et al. 2012; Margres et al. 2013). From *Colubridae* family, *Boiga irregularis* has a TCTP sequence on the database, although it is not mentioned in the reference manuscript (McGivern et al. 2014). The annotation of TCTP from *Python bivittatus* (Burmese python) was derived from a genomic sequence using gene prediction method. The search for TCTP

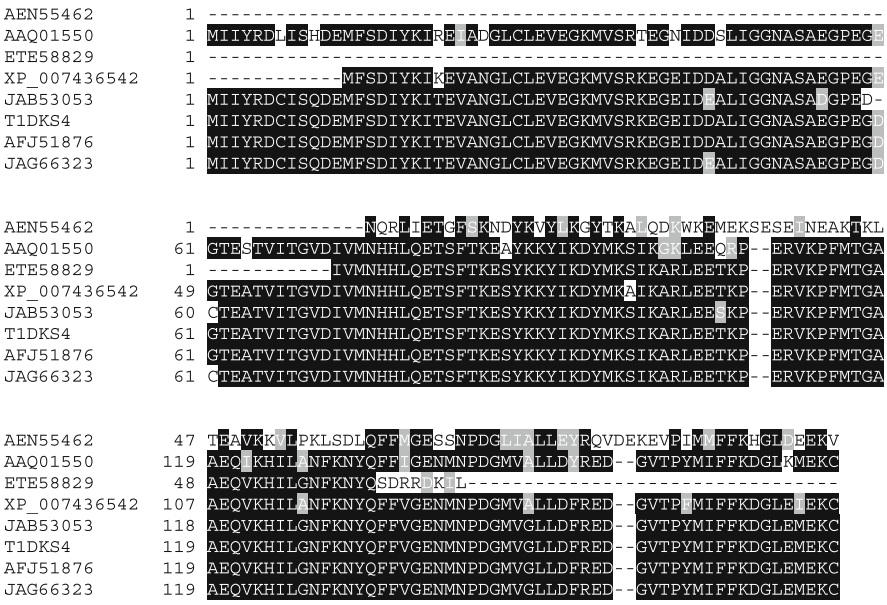

Fig. 9.2 Multiple alignment of TCTP protein sequences from different species of snakes compared to human and *Loxosceles intermedia* TCTP. Sequence alignment was performed using the Clustal Omega program and formatted with the BOXSHADE program (version 3.21). Fully conserved positions are shaded in *black* and conservative substitutions are in *gray*. *Loxosceles intermedia* TCTP (AEN55462.1); *Homo sapiens* TCTP (AAQ01550.1); *Ophiophagus hannah* (ETE58829.1); *Python bivittatus* (XP_007436542.1); *Micrurus fulvius* (JAB53053.1); *Crotalus horridus* (T1DKS4.1); *Crotalus adamanteus* (AFJ51876.1); *Boiga irregularis* (JAG66323.1)

sequences on the scorpions' database results in no matches; however, there is at least 6700 sequences from scorpions on the PUBMED protein database.

9.4 Perspectives

Worldwide over 44,900 species of spiders have been identified, but only a handful of venoms from these species have been characterized with molecular techniques (Platinick 2014). TCTP has already been described in gland secretions of many other arthropods, mainly parasites, such as ixodid ticks (Mulenga and Azad 2005) and the lamprey *Lampetra japonica* (Sun et al. 2008). Thus, it is likely that TCTP's activity as a histamine releasing factor plays a role in inflammation and infection processes of venomous accidents and parasitic conditions pathophysiology, but its primary role as a venom component still needs be elucidated.

References

Buch DR, Souza FN, Meissner GO, Morgon AM, Gremski LH, Ferrer VP, Trevisan-Silva D, Matsubara FH, Boia-Ferreira M, Sade YB, Chaves-Moreira D, Gremski W, Veiga SS, Chaim OM, Senff-Ribeiro A (2015) Brown spider (*Loxosceles* genus) venom toxins: evaluation of biological conservation by immune cross-reactivity. Toxicon 108:154–166

da Silva PH, da Silveira RB, Appel MH, Mangili OC, Gremski W, Veiga SS (2004) Brown spiders and Loxoscelism. Toxicon 44:693–709

dos Santos VL, Franco CR, Viggiano RL, da Silveira RB, Cantao MP, Mangili OC, Veiga SS, Gremski W (2000) Structural and ultrastructural description of the venom gland of *Loxosceles intermedia* (brown spider). Toxicon 38:265–285

Escoubas P, Rash L (2004) Tarantulas: eight-legged pharmacists and combinatorial chemists. Toxicon 43(5):555–574

Gremski LH, Da Silveira RB, Chaim OM, Probst CM, Ferrer VP, Nowatzki J, Weinschutz HC, Madeira HM, Gremski W, Nader HB, Senff-Ribeiro A, Veiga SS (2010) A novel expression profile of the *Loxosceles intermedia* spider venomous gland revealed by transcriptome analysis. Mol BioSyst 6(12):2403–2416

Gremski LH, Trevisan-Silva D, Ferrer VP, Matsubara FH, Meissner GO, Wille AC, Vuitika L, Dias-Lopes C, Ullah A, De Moraes FR, Chávez-Olórtegui C, Barbaro KC, Murakami MT, Arni RK, Senff-Ribeiro A, Chaim OM, Veiga SS (2014) Recent advances in the understanding of brown spider venoms: from the biology of spiders to the molecular mechanisms of toxins. Toxicon 83:91–120

Kimura T, Ono S, Kubo T (2012) Molecular cloning and sequence analysis of the cDNAs encoding toxin-like peptides from the venom glands of tarantula *Grammostola rosea*. Int J Peptides 2012:731293. https://doi.org/10.1155/2012/731293

MacDonald SM (2012) Potential role of histamine releasing factor (HRF) as a therapeutic target for treating asthma and allergy. J Asthma Allergy 5:51–59

Margres MJ, Aronow K, Loyacano J, Rokyta DR (2013) The venom-gland transcriptome of the eastern coral snake (*Micrurus fulvius*) reveals high venom complexity in the intragenomic evolution of venoms. BMC Genomics 14:531. https://doi.org/10.1186/1471-2164-14-531

McGivern JJ, Wray KP, Margres MJ, Couch ME, Mackessy SP, Rokyta DR (2014) RNA-seq and high-definition mass spectrometry reveal the complex and divergent venoms of two rear-fanged colubrid snakes. BMC Genomics 15:1061. https://doi.org/10.1186/1471-2164-15-1061

Mulenga A, Azad AF (2005) The molecular and biological analysis of ixodid ticks histamine release factors. Exp Appl Acarol 37:215–229

Paludo KS, Biscaia SM, Chaim OM, Otuki MF, Naliwaiko K, Dombrowski PA, Franco CR, Veiga SS (2009) Inflammatory events induced by brown spider venom and its recombinant dermonecrotic toxin: a pharmacological investigation. Comp Biochem Physiol C Toxicol Pharmacol 149(3):323–333

Platinick NI (2014) The world spider catalog. American Museum of Natural History, New York, NY. Version 14.5

Rattmann YD, Pereira CR, Cury Y, Gremski W, Marques MC, da Silva-Santos JE (2008) Vascular permeability and vasodilation induced by the *Loxosceles intermedia* venom in rats: involvement of mast cell degranulation, histamine and 5-HT receptors. Toxicon 51(3):363–372

Ribeiro RO, Chaim OM, da Silveira RB, Gremski LH, Sade YB, Paludo KS, Senff-Ribeiro A, de Moura J, Chávez-Olórtegui C, Gremski W, Nader HB, Veiga SS (2007) Biological and structural comparison of recombinant phospholipase D toxins from *Loxosceles intermedia* (brown spider) venom. Toxicon 50(8):1162–1174

Rokyta DR, Lemmon AR, Margres MJ, Aronow K (2012) The venom-gland transcriptome of the eastern diamondback rattlesnake (*Crotalus adamanteus*). BMC Genomics 13:312. https://doi.org/10.1186/1471-2164-13-312

Rokyta DR, Wray KP, Margres MJ (2013) The genesis of an exceptionally lethal venom in the timber rattlesnake (*Crotalus horridus*) revealed through comparative venom-gland transcriptomics. BMC Genomics 14:394. https://doi.org/10.1186/1471-2164-14-394

Sade YB, Bóia-Ferreira M, Gremski LH, Da Silveira RB, Gremski W, Senff-Ribeiro A, Chaim OM, Veiga SS (2012) Molecular cloning, heterologous expression and functional characterization of a novel translationally-controlled tumor protein (TCTP) family member from *Loxosceles intermedia* (brown spider) venom. Int J Biochem Cell Biol 44(1):170–177

Sun J, Wu Y, Wang J, Ma F, Liu X, Li Q (2008) Novel translationally controlled tumor protein homologue in the buccal gland secretion of *Lampetra japonica*. Biochimie 90:1760–1768

Vetter RS (2008) Spiders of the genus Loxosceles (*Araneae, Sicariidae*): a review of biological, medical and psychological aspects regarding envenomations. J Arachnol 36:150–163

Zhang Y, Chen J, Tang X, Wang F, Jiang L, Xiong X, Wang M, Rong M, Liu Z, Liang S (2010) Transcriptome analysis of the venom glands of the Chinese wolf spider *Lycosa singoriensis*. Zoology (Jena) 113(1):10–18. https://doi.org/10.1016/j.zool.2009.04.001

Zobel-Thropp PA, Correa SM, Garb JE, Binford GJ (2014) Spit and venom from *Scytodes* spiders: a diverse and distinct cocktail. J Proteome Res 13:817–835. https://doi.org/10.1021/pr400875s