ANIMAL TOXINS: STRUCTURE, FUNCTION AND BIOTECHNOLOGICAL APPLICATIONS

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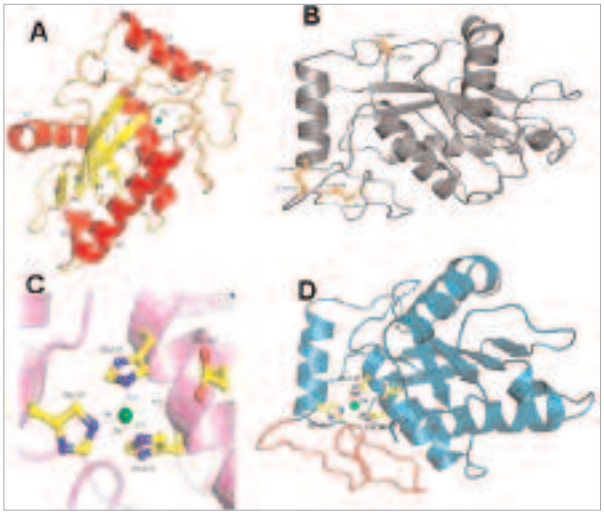
Animal toxins have contributed significantly for the development of Biological and Biomedical Sciences. These molecules, used as important tools in the investigation of cellular and molecular mechanisms, are involved in immunological, pharmacological and toxicological processes. In addition, they constitute interesting molecular models for the development of biotechnological strategies to generate therapeutic agents and/or experimental tools for basic and applied research. However, animal venoms/toxins still lack additional biological and/or functional characterization, including those from snakes, toads and scorpions. Within this purpose, the isolation and biochemical structural and functional characterization of biologically active proteins/components of these venoms will be able to provide important information for a better understanding of the composition and physiopathological effects of these toxins. This project aims at the functional and/or structural analyses of new toxins and model toxins (already described) from snakes (*Bothrops jararacussu*, *B. pirajai*, *B. alternatus*, *B. atrox* e *Crotalus d. terrificus*), scorpion (*Tityus serrulatus*) and toad (*Bufo paracnemis*). Isolation of biolocally active components will make use of classical chromatographic techniques, such as gel filtration, ionic exchange, hydrophobic interaction, bioaffinity and HPLC (reversed phase). This phase of the project is fundamental for all the following ones to be developed, since it will provide the active components which, along with the crude venom, will be objects of study of this project. The investigation of the biological activities of venoms and toxins will be multiparametric, considering the adequation of the assays to the character of toxins (new or models). Effects still roughly explored of these toxins will be evaluated on the immune system (complement, apoptosis and inflammation), microorganisms (leishmanicide, trypanocide, bactericide, fungicide and antiviral activities) and cell lines (cytotoxicity, antitumoral effect and apoptosis).

Actions upon Ca^{2+} and Na^{+} channels and upon receptor and transporter systems for L-glutamate and GABA will be explored as well. In addition, activities as hyaluronidase, proteolytic, PLA2, L-amino acid oxidase, hemorrhagic, myotoxic, edema inducing, coagulant, lectinic and anticoagulant will also be evaluated. These activities were chosen since they are target of the venoms actions and highly relevant in physiological processes. Within the different systems to be evaluated, the structure of the isolated toxins will be the basis for the possible elucidation of the structure – function relationship.

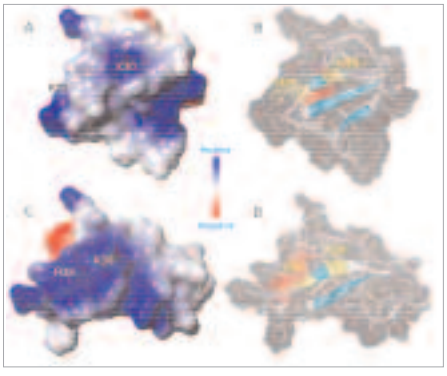


Structure of B. jararacussu *metalloprotease BJUSSUMP-II. (A): Secondary and tertiary structure of the BJUSSUMP-II model. The ion Zn^{2+} is shown as a green sphere. (B): Disulfide bridges (represented as sticks) present in the BJUSSUMP-II model. (C): Distances between the ion Zn^{2+} (green sphere) and the residues from the catalytic site of the BJUSSUMP-II model. (D): Cartoon representation of BJUSSUMP-II model highlighting the flexible region 153-176 (in red). (MARCUSSI et al., 2007).*

The structural characterization of the toxins will be achieved by automatic sequencing, X-ray crystallography and molecular modeling. Considering the number of venoms and toxins to be explored and the proposed assays for characterizations of their effects and structures, we believe that this project is multidisciplinary, comprehensive and promising. The results of the proposed project will broaden the understanding of the structure/function relationship of toxins in biological systems, which will contribute for the generation of new tools for basic or applied research.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

L-Aminoacido oxidases (LAAOs) isolated from the venom of *B. jararacussu*, *B. moojeni* and *B. pirajai*, were characterized biochemically, functionally and structurally. From the venom of *B. jararacussu*, two metalloproteases were isolated: BJUSSUMP-I, Mr ~ 60,000 and pI 5.6; and BJUSSUMP-II, Mr ~ 24,000 and pI 6,43. Both metalloproteases have been proved potent αβ-fibrinogenases, and inhibit platelet aggregation. A serinoprotease, BjussuSP-I, was also isolated, and exhibits thrombin-like proteolytic activity. It also presented pro-coagulant and potential calicrein-like dysfibrinogening activities, the latter likely to be of clinical relevance. Also isolated were: phospholipases A2 called Bmoo-I-PLA2 and Bmoo-II-PLA2 from *B. moojeni*; acidic Bp-PLA2 from *B. pauloensis*; and two basic neurotoxic isoforms from *B. neuwiedi pauloensis*. MjTX-II from *B. moojeni* complexed with fatty acid had both its tertiary and quaternary structures elucitadated. The PLA2 (CB)-crotoxin complex from the venom of *C. d. terrificus* was crystallized. NMR studies with the venom of scorpion *Tityus serrulatus* allowed for the structure determination of alpha-KTx12.1, a toxin that blocks potassium channels. They were also tested with crude venom, and the toxins TsTX-I and TsTX-V, with resulting data showing that they induce a marked increase both in blood pressure and in plasmatic catecholamines. Also from the venom of *Tityus serrulatus*, proteases that are able to activate the complement system could be identified. An acidic protein able to activate the complement system has also been isolated from the venom of toad *Bufo paracnemis*. The results obtained to date should contribute significantly to the development of Toxinology in Brazil, improving the understanding of the mechanism of action of proteins and toxins from snake venoms, thus being valuable for searching pharmacologically active new molecules of interest in the clinical-medical and scientific fields of knowledge.



Accessible surfaces of the last structures of each MD simulation of the pH 7.0 (A and B) and 4.0 (C and D). Areas represented in A and C are colored according to the electrostatic potential. Waste His28 and Lys30, are highlighted in yellow (B and D)

MAIN PUBLICATIONS

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