

Spider Silk as a Source of Novel Bioactive Metabolites: a Search for Factors with Antimicrobial and Inhibitory Activities

Bisetto SP^{1,2}, Silva Junior P¹.

¹Laboratório Especial de Toxinologia Aplicada/CAT-CEPID - Instituto Butantan, São Paulo/SP, Brasil; ²Instituto de Biociências – USP, São Paulo/SP, Brasil

Introduction: Spider silk is a remarkably strong material with tensile strength comparable to high-grade steel. It is used by the spider in many different ways, including for capturing prey, production of egg sacs and construction of shelter. Although different species of spider have different types of silk, a general trend in spider silk structure is a sequence of amino acids self-assembled into a beta sheet conformation, alternated by segments of amino acids with bulky side-groups. Due to its unique properties, this material has been for a long time used by humans, for instance the silk from *Nephila clavipes* has been used for many years as material for the treatment of wounds due to its antiseptic properties. The identification of the molecules responsible for some of these properties of the spider silk may be of great pharmaceutical importance. **Objectives:** Investigate the presence of antibiotic and non antibiotic – enzyme inhibitory – bioactive molecules among the spider silk proteins from the spiders *Nephilengys cruentata*, *Nephila clavipes* (ARANEAE, NEPHILIDAE) e *Avicularia juruensis* (ARANEAE, THERAPHOSIDAE), as the probable causes for several of its unique properties. **Methods:** The silk from each spider was obtained in two different forms: directly from the animal by mechanically induced production, or through web collection from its habitat. Extracts of the silk were tested for antimicrobial activity through liquid growth inhibition assay against *Candida albicans*, *Micrococcus luteus* and *Escherichia coli*, and for enzyme inhibitory activity in enzyme inhibition assays using trypsin and endopeptidase 24.15 (EC 3.4.24.15; EP 24.15). Purification of the material was held through its application in reversed-phase chromatography (RP-HPLC), using a Jupiter semi preparative C18 column. The column effluent was monitored by absorbance at 225 nm and the activities were determined once again by liquid growth inhibition assay and enzyme inhibition assay. For further purification, when necessary, the fractions were applied to reversed-phase chromatography (RP-HPLC), using a Jupiter analytic C18 column. All fractions with activity, when purified, will be sent to mass spectrometry for further analysis. **Results:** Antimicrobial factors with activity against yeast, gram positive and gram negative bacteria (*Candida albicans*, *Micrococcus luteus* and *Escherichia coli*) were found in the non purified and purified material obtained from two of the spiders. These factors will be sequenced and their action spectra determined. In the analysis of the enzyme inhibitory activity some fractions with activity against trypsin were found and will be sent to mass spectrometry for further analysis. Fractions with EP 24.15 inhibitory activity are still being identified and in process of purification.

Palavras-chave: antimicrobial activity, enzyme inhibition, spider silk
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