Detection of Distinct Metalloproteinases in the Venom of *Bothrops neuwiedi* Snake

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The SVMPs are responsible for most of the symptoms of *Bothrops* snakes envenoming, acting on different targets of hemostasis. In this work, our aim is to demonstrate the diversity of SVMPs in the venom of *B. neuwiedi*. Venom was obtained from snakes kept in captivity at Butantan Institute. The venom was fractionated by HPLC (Agilent) in C-18 column or by HiPrep 16/60 Sephacryl S-200 followed by Mono-Q 5/5 columns (Äkta, GE). The fractions were analyzed by SDS-PAGE and by the reactivity with specific antibodies anti-SVMPs by dot-blot. Fractionation of the venom by HPLC showed the existence of at least six different SVMPs. To isolate these SVMPs, the venom fractionated by column Hiprep followed by Mono-Q allowed the identification of at least two distinct fractions with SVMPs class P-III with different hemorrhagic activity, and two fractions with SVMPs class P-I. To demonstrate the activity of the venom on blood coagulation, the crude venom and isolated fractions were tested. Significant decrease of the clotting time was observed after addition of 10 ng of crude venom. Isolated fractions showed distinct activities on blood clotting. Analyzing the data, we could see the high diversity of SVMPs in the venom of *B. neuwiedi*, which presented strong procoagulant activity. We demonstrated the presence of metalloproteinase from classes P-I and P-III. Our data indicate the structural diversity of SVMPs and suggest that different components of coagulation system may be targeted by these toxins, thus accounting for the strong procoagulant activity of *B. neuwiedi* venom.

Word Keys: *Bothrops neuwiedi*, metalloproteinases.
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