USING DM43 AND DM64, TWO ANTITOXINS FROM DIDELPHIDAE, TO STUDY THE SNAKE VENOM SUB-PROTEOMES

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Snake venoms are complex mixtures of proteins and peptides with different biological activities, many of them very toxic. Some animals, including the opossum *Didelphis auritas*, are resistant to snake venoms due to the presence of neutralizing factors in their blood. Two natural inhibitors have been isolated from opossum serum, DM43 and DM64 with antihaemorrhagic and antimyotoxic activities, respectively. They inhibit snake venom metalloproteinases and myotoxins through non-covalent complex formation with these proteins. In this study, we have used DM43, DM64 and proteomic techniques to explore snake venom subproteomes. Several venoms were chromatographed through an affinity column containing immobilized DM43 or DM64. Bound and unbound fractions were analyzed either by SDS-PAGE and/or 2D-PAGE, followed by identification using MALDI-TOF/TOF mass spectrometry. Following this methodology, we could classify venoms from *Bothrops alternatus*, *B. asper*, *B. atrox*, *B. insularis*, *B. jararaca*, *B. jararacussu*, *B. moojeni*, *B. neuwiedi*, *Crotalus adamanteus*, *C. atrox*, *C. durissus terrificus*, L*achesis muta muta* and *Naja naja atra* according to their relative content of metalloproteinases (PI, PIII and/or their fragments) using DM43. Venom fractions not bound to DM43 column were equally analyzed and were composed basically of serine proteases, phospholipase A_2, C-type lectins, L-amino acid oxidases, nerve growth factor, and/or some metalloproteinases and unidentified spots. On the other hand, snake venoms of *B. asper*, *B. jararacussu*, *B. neuwiedi*, *B. moojeni* and *B. jararaca* were analyzed using DM64 affinity column. Bound venom fractions were composed basically of phospholipase A_2, serine proteinase and C- type lectins. So far, just not bound fractions of venoms of *B. asper* and *B. jararaca* were analyzed and were composed, mainly by metalloproteinases and some cysteine-rich secretory protein (CRISP), phospholipase A_2 and serine proteinases. Studied venoms presented important proteic variability, with frequent detection of multiple forms of the same protein and several members of the same protein family. DM43 and DM64 can be very useful as a tool for analyze the complexity of snake venoms and in the search for new molecules, and they can also be used to better understand the mechanism of action of these toxins in the envenomation. DM43 and DM64 affinity chromatography associated with proteomic techniques showed to be useful tools to separate and identify proteins from snake venoms.

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