Interaction Of Antimyotoxin DM64 With Snake Venom Proteins Analyzed By Proteomics Technology

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Envenomation by snakes still represents a serious public health problem in tropical developing countries. In particular, bothropic venoms are known to induce important local tissue damage, such as hemorrhage and myonecrosis. The opossum *Didelphis aurita* is highly resistant to bothropic snake venoms due to the presence of different serum neutralizing factors, such as DM43 and DM64. This last antitoxin is a 64 kDa acidic protein with 15% glycosylation, which inhibits both the *in vivo* myotoxicity and the *in vitro* cytotoxicity of myotoxins I and II from *Bothrops asper* venom, but does not inhibit the phospholipase A_2 activity of myotoxin I. The present work aimed to analyze the interaction between DM64 and proteins from different snake venoms. Non-saturating amounts of venoms from *Bothrops asper*, *Bothrops jararacussu*, *Bothrops neuwiedi*, *Bothrops moojeni* and *Bothrops jararaca* were separately chromatographed through an affinity column coupled with homogeneous DM64. Bound fractions were analyzed by uni- and two-dimensional gel electrophoresis, followed by identification by MALDI-TOF/TOF mass spectrometry. All venom fractions bound to DM64 column were enriched in phospholipases A_2. To a lesser extent, they also showed the presence of serine proteinases and C-type lectins. Using this approach, we could easily visualize and compare the myotoxin venom subproteomes from different snakes of the *Bothrops* genus. Non-bound fractions from *B. asper* and *B. jararaca* venoms were mainly composed of several metalloproteinases, serineproteinases, cysteine-rich secretory proteins (CRISP), a few phospholipases A_2 and several unidentified spots. DM64 affinity chromatography associated with proteomics techniques showed to be useful tools to separate and identify proteins from snake venoms, contributing to a better comprehension of venom heterogeneity.

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