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Structural and phylogenetic studies with a theoretical model of the BjussuMP-I, a metalloprotease isolated from the Bothrops jararacussu venom

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Short Abstract: In this work the phylogenetic and structural analyses of a theoretical BjussuMP-I metalloprotease/catalytic domain model were performed to get new insights into the molecular evolution of the metalloproteases and to identify eventual structural differences responsible for the several biochemical activities carried out by these enzymes.

Long Abstract:
Snake venom metalloproteases (SVMPs) comprise zinc-dependent enzymes members of the Reprolysin subfamily, which include the ADAMs (A Disintegrin And Metalloproteinase) domains. They are responsible for a relevant pathophysiology in envenomation, including local and systemic hemorrhage. Alteration of basal membrane components and interactions with some factors of the coagulation pathway are the main biochemical actions of these classes of enzymes. Moreover, these compounds are also important tool to understand signaling mechanisms involved in apoptosis and cell adhesion alterations related to wound healing and tumoral metastasis. Recently, the BjussuMP-I, a 60 kDa hemorrhagic metalloprotease, was isolated from the Bothrops jararacussu snake venom and sequenced by our research group. In order to find out new insights into the molecular evolution of the metalloproteases, a phylogenetic analysis of the BjussuMP-I metalloprotease/catalytic domain was performed. This phylogenetic analysis was performed in two parts: i) the study of the evolutionary origins of the SVMPs and ii) the search for possible relations between these toxins and other homologue proteins. Initially, a phylogenetic tree was built to determine the evolutionary relations between the SVMPs and the cellular metalloproteases from other organisms, using sequences selected in the NCBI and Pfam databases. The other homologue sequences were found in animals, fungi, and in the Bacillus licheniformis (strain dsm 13) (UniProt/TrEMBL|Q65DR9), a well-known soil bacterium used for biotechnological applications. The analysis of the first phylogenetic tree shows there is an unequivocal relation between the SVMPs and other metalloproteases identified in different species. Thus, despite of all metalloproteases selected here are taking part in the reprolysin family, some molecules from Xenopus laevis, Danio rerio, Gallus gallus, Mus musculus, and Homo sapiens are more related to the SVMPs than others. Based on this information, it was possible to classify the sequences in four groups (viperid SVMPs, elapid SVMPs,

proto-SVMPs, and non-SVMPs), according their phylogeny and phenotype. The analysis of this philogenetic tree shows that the evolutionary history of the proto-SVMP has begun with a primitive gene duplication occurred before the emergence of the common ancestor of teleosts and tetrapods. The second part of this phylogenetic analysis was executed with the catalytic domain sequences of all SVMPs selected in the NCBI and UniProt databases. In this analysis was not possible to arrange these metalloproteases in separated branches according with their capacity of inducing hemorrhage. Analyzing this phylogenetic tree is also possible to suggest that the hemorrhagic activity was a biochemical activity present in the first SVMPs due to the higher number of hemorrhagic toxin sequences found in the databases. Apparently, some of these toxins lost this activity during their processes of molecular evolution. The reasons for the lack of hemorrhagic action are not clear and could be related to new roles played by these non-hemorrhagic SVMPs. Furthermore, a more detailed analysis of this phylogenetic tree shows a remarkable separation between the Viperidae family species and those from other groups. After the phylogenetic analysis, the initial model of the BjussuMP-I metalloprotease domain was generated through folding recognition (threading) techniques. All hypothetical structures generated were analyzed and the better value of Z-score (49.05) was selected. Then, the selected model obtained by threading was submitted to a molecular dynamics (MD) simulation in order to improve its stereochemical quality, using the GROMACS program (Groningen Machine for Chemical Simulation) package version 3.2.1. The analysis of the final model showed it is very similar with other three-dimensional BjussuMP-I catalytic domains already described, presenting an ellipsoidal form and two subdomains. The major subdomain is constituted by the first 152 residues and presents four α-helices and five stranded β-sheets, while the last 98 residues belong to the minor subdomain, which is formed by one α-helix and several loops. The analysis of the alignment of all metalloprotease catalytic domain sequences using the program Chimera showed few surface residues were conserved during the evolution, in contrast with the internal residues which remained practically unaltered. Additionally, the catalytic domain sequences from viperid and elapid SVMPs and other homologue sequences were separated in four groups according to phylogeny and phenotype and aligned separately. An interesting feature was then revealed about the catalytic domain of the metalloproteases: each group presented a determined set of surface conserved residues. All sets are composed by surface conserved residues present in all sequences, however there are additional surface conserved residues specifically found in each group. A common characteristic of the molecular surfaces of all metalloproteases aligned, which was not changed during the evolution history of these proteins, is the presence of large hydrophobic areas. However, the most remarkable structural difference between the catalytic domains of the metalloproteases seems to be related to the type and number of the specific electric-charged surface residues present in each group. This fact suggests that these charged surface residues may play an important role in the specific biochemical reactions executed by each group of metalloproteases. Another interesting structural feature that could be a hint to explain the different biological roles of the SVMP catalytic domains and their more related sequences (proto-SVMPs) is found in the positions 15 and 21. In the viperid SVMP catalytic domains, these positions are occupied by positive-charged surface conserved residues (Arg15 or His15 and Lys20), whereas in the proto-SVMPs these positions are occupied only by non-charged residues (Ala15 and Phe20). The elapid SVMPs sequences also present a clear predominance of positive-charged residues at these same positions. Thus, these positive-charged residues may be essential for the biochemical functions played only by the SVMPs. In conclusion, interesting structural features were

identified for the SVMPs and their more related metalloproteases: these molecules are characterized by the presence of large hydrophobic areas and group-specific charged conserved residues (mainly positive-charged residues) at their surfaces. Therefore, these characteristics are probably important for the interaction between these toxins and their substrates.