Natural inhibitors of snake venom toxins: a promising source of therapeutic peptides for toxin-centric next-generation antivenoms

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By 2030, the World Health Organization aims to halve the global rates of death and disability caused by snake envenomation. To meet this challenge, a comprehensive strategy has been recently devised, which includes the need to accelerate research into new therapies. In this context, the use of effective inhibitors of tissue-damaging toxins [phospholipase A2 (PLA2) and metalloendopeptidase (SVMP)] constitutes an attractive adjunctive therapy. Circulating antitoxins observed in animals naturally resistant to snake envenomation represent a rich source of molecular scaffolds for developing novel peptide-based therapeutics. This study aimed to characterize the interaction between the anti-hemorrhagic protein DM43 from the opossum Didelphis aurita (SisGen AF0A111, CEUA/IOC L-035/2018) and the SVMP BaP1 from Bothrops asper venom, using an integrated Structural Biology approach. The stoichiometry of the interaction between DM43 and BaP1 was analyzed using sedimentation velocity analytical ultracentrifugation. The toxin-antitoxin complex was stabilized with BS3 [(bis(sulfosuccinimidyl)suberate], and all cross-linked peptides (intra- and inter-proteins) were identified by nLC-MS/MS on the QExactive HF-X mass spectrometer, using the SIM-XL software, followed by manual validation. The five best DM43 models generated by AlphaFold2 were evaluated according to the level of global validation of the cross-links, using the Topolink software to measure the topological distances between the cross-linked residues identified (Lys or Ser). Molecular dynamics simulation strategies were also applied to evaluate the stability of the models and the rate of cross-linking validation as a function of time. DM43 formed a non-covalent equimolar complex with BaP1, inhibiting its proteolytic and hemorrhagic activities. DM43 structural models revealed a low validation of intra-DM43 cross-links, indicating that the computational models do not accurately reflect the conformational structure of the inhibitor in solution. Most inter-protein links observed involved the third domain of DM43 and the regions of BaP1 between residues 60-80 and 150-170. These distance constraints will be used to guide the molecular docking of the crystal structure of BaP1 with an optimized model of DM43. With these strategies, we intend to topologically characterize the toxin-antitoxin complex, defining the regions of DM43 potentially involved in its anti-ophidic properties.

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