In silico structural studies of phospholipases A_2 inhibitors from snake blood

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Several snake species possess endogenous phospholipase A_2 inhibitors (PLIs) in their blood plasma, which their primary role is protection against an eventual presence of toxic phospholipase A_2 (PLA_2) from their venom glands in the circulation. These inhibitors have an oligomeric structure of, at least, three subunits and have been categorized into three classes (α, β and γ) based on characteristic structural features. In the present work, we constructed *in silico* models of the three classes of PLIs from South American snakes by threading modelling using Phyre2 server and molecular dynamics simulations using GROMACS v.4.5.3 software in GROMOS 96 53a6 force field; starting from sequenced or deduced amino acid sequences. The model of αPLI from *Bothrops alternatus* (named BaltMIP) presented the typical features of C-type lectin domains under monomeric configuration: an α-helical neck and carbohydrate recognition domain (CRD). We also constructed the *in silico* model of BaltMIP trimer by C_α atom alignments between the final αPLI model and the monomers of the homologue trimeric human lung surfactant protein D (SP-D) and simulated annealing simulations. Structural analysis of the BaltMIP trimer confirmed that α-helical neck is essential for trimer stabilization. Besides, CRD domains form a negatively charged central pore, which is actually the binding site for acid PLA_2. The βPLI model, based on translated aminoacid sequence of a βPLI transcript isolated from *Bothrops jaracussu* liver, presented the characteristic tandem leucine-rich repeats (LRRs) in its structure. These LRRs are rich on positively charged residues that could constitute the binding site for basic PLA_2. Finally, the γPLI model from *Crotalus durissus terrificus* (named CNF, standing *Crotalus* neutralization factor) displayed well-defined three-finger domains in its tertiary structure. Besides, structural analysis of CNF *in silico* model combined to experimental data showed that tyrosine residues could play an important role in the oligomerization of CNF.

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