

CLONING AND SEQUENCE ANALYSIS OF GYROXIN-LYKE FROM *Crotalus durissus terrificus* VENOM GLAND

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Gyroxin is an important toxin from *C. d. terrificus* venom however it is only partially characterized until now; it is a multifunctional toxin with thrombin-like activity and induces the barril rotation syndrome. A venom gland cDNA library from a single *C. d. terrificus* specimen was amplified with specific primers. The PCR product was cloned and sequenced. Gyroxin was synthesized as a prozymogen which 18 amino acids as a signal peptide, a propeptide of 6 and a matured protein with 238 amino acids. A clone with 895 bp length composed by a mature toxin-coding region of 717 bp and a 3'UTR of 178 bp length with a polyadenilation signal and poliA(+) tail. The predicted primary sequence of matured peptide displayed a relatively high amino acid homology to others members of the snake venom serine protease family (SVSPs) such as 92% similarity with crotalase and 82% with gyroxin analog (*Lachesis muta*). It contains 12 conserved cysteins, which form 6 SS bounds by similarity. A cluster analysis of an alignment with 32 typical SVSPs (including the gyroxin-like clone) generated a functional dendrogram organized in three major clusters: one with thrombin-like activity, other with kininogenase and the third one with plasminogen activators. The presumed sequence of mature gyroxin is close related to toxins from thrombin-like group. A three-dimensional model of gyroxin was built by homology modeling using TSV-PA (1BKY) crystal structure and AAV-SP-I (1OP0) and AAV-SP-II (1OP2) as template. Gyroxin model is useful to compare with thrombin and other SVSPs. The gyroxin cDNA sequence coding to mature toxin has been cloning into a dicistronic vector pED-gyroxin and expressing by mammalian cells CHO.DHFR-. Almost three others serine proteases are cloned and it will be analyzed.

KEY WORDS: gyroxin, *Crotalus*, serine protease, cloning, snake venom

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