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Purification, characterization, crystallization and theoretical molecular modeling of gyroxin fraction from *Crotalus durissus terrificus* venom (Laurenti, 1768)

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ABSTRACT: The venom of *Crotalus durissus terrificus* snakes presents various substances, including a serine protease with thrombin-like activity, called gyroxin, that clots plasmatic fibrinogen and promote the fibrin formation. The aim of this study was to purify and structurally characterize the gyroxin enzyme from *Crotalus durissus terrificus* venom. For isolation and purification, the following methods were employed: gel filtration on Sephadex G75 column and affinity chromatography on benzamidine Sepharose 6B; 12% SDS-PAGE under reducing conditions; N-terminal sequence analysis; cDNA cloning and expression through RT-PCR and crystallization tests. Theoretical molecular modeling was performed using bioinformatics tools based on comparative analysis of other serine proteases deposited in the NCBI (National Center for Biotechnology Information) database. Protein N-terminal sequencing produced a single chain with a molecular mass of ~30 kDa while its full-length cDNA had 714 bp which encoded a mature protein containing 238 amino acids. Crystals were obtained from the solutions 2 and 5 of the Crystal Screen Kit®, two and one respectively, that reveal the protein constitution of the sample. For multiple sequence alignments of gyroxin-like B2.1 with six other serine proteases obtained from snake venoms (SVSPs), the preservation of cysteine residues and their main structural elements (alpha-helices, beta-barrel and loops) was indicated. The localization of the catalytic triad in His57, Asp102 and Ser198 as well as S1 and S2 specific activity sites in Thr193 and Gli215 amino acids was pointed. The area of recognition and

cleavage of fibrinogen in SVSPs for modeling gyroxin B2.1 sequence was located at Arg60, Arg72, Gln75, Arg81, Arg82, Lis85, Glu86 and Lis87 residues. Theoretical modeling of gyroxin fraction generated a classical structure consisting of two alpha-helices, two beta-barrel structures, five disulfide bridges and loops in positions 37, 60, 70, 99, 148, 174 and 218. These results provided information about the functional structure of gyroxin allowing its application in the design of new drugs.

KEY WORDS: purification, characterization, crystallization, theoretical modeling, gyroxin.

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