MOLECULAR MECHANISM OF ANTI-BLOOD COAGULATION TARGETING PLASMA FACTORS IXA AND XA BY A GALACTOSIDE-BINDING LECTIN FROM CERASTES CERASTES SNAKE VENOM

[1]CHÉRIFI F., [2]SAOUD S. , [3]LARABA-DJEBARI F.

USTHB, Faculty of Biological Sciences; Laboratory of Cellular and Molecular Biology, BP 32 El-Alia, Bab Ezzouar, Algiers, Algeria.
E-mail: [1]flaraba@hotmail.com. flaraba@usthb.dz

Objective - A Ca^{2+}-dependent type (C-type) galactoside-binding lectin designated Cc-Lec was purified from venom of the snake Cerastes cerastes by D-lactose-Sepharose affinity column chromatography. Cc-Lec is a disulfide-linked homodimer composed of 17-kDa subunits. The 160-residue amino acid sequence of each subunit was determined by a combination of tandem mass spectrometry and multiple sequence alignment, therefore, it shared high similarities with many C-type lectins from snake venoms and appeared to belong to the animal C-type lectin family. This study aimed to elucidate the molecular mechanism by which this lectin acted to exhibit its potential anti-coagulation.

Materials and Methods - Sequence of Cc-Lec homologous from other species were retrieved from NCBI databases using the BLAST program,. Sequence alignments between Cc-Lec and their homologous were analyzed using ClustalW program and visualized by ESPript 3.0. 3D homology model of Cc-Lec was built with Prime in Schrödinger. An initial 3D Cc-Lec structure was then built using homology modeling and was subsequently optimized with protein refinement tools included in prime. The docking protein-protein requires the Piper program, included in the BioLuminate tool included in Schrödinger.

Results - In silico study of structural properties of Cc-Lec showed a modeled 3D structure appearing as homodimer cross-linked by one disulfide bridge on Cys_{83} with no glycosylation. This optimized 3D structure was built with 1% gaps, an identity of 48%, a positivity of 61% and an alignment score of 317, the sequence of 1J35_B.pdb has the best homology in the BLAST search for Cc-Lec on proteins in PDB. Multiple alignment of Cc-Lec with other snake venom C-type lectins revealed a highly conserved peptide among these proteins and many conserved amino acid residues such as Cys^{37}, Cys^{54}, Leu^{63}, Gly^{92}, Trp^{141}. Conserved cysteines can be related to intramolecular disulfide linkages, while the other amino acids may be involved in carbohydrate recognition and lectin's interaction with several ligands. According to obtained results, when analyzed with related snake venom proteins; Cc-Lec was grouped in the same cluster with two C-type lectins derived from Viperidae's venom. The lectin strongly agglutinated human erythrocytes which when subjected to ligand screening, both mono- and disaccharides, only D-lactose with β-galactoside inhibited this activity in presence of calcium. The hemagglutinating activity of Cc-Lec is optimum at pH 7.0–12.0, stable up to 60°C, and dependent on divalent cations. Further, Cc-Lec exhibited anticoagulation through specific binding to coagulation factors IX and X. All these experimental data were reinforced by molecular docking studies. Lactose interacts with Cc-Lec complexed with calcium by hydroxyl groups in positions 3 and 4 of galactose which is directly linked to the lectin by forming hydrogen bonds with the residues Lys^{44}, Lys^{47} and Lys^{87}. Glucose also interacts with residues of Ser^{33} and Lys^{87} of the lectin by hydrogen bonds.

Conclusions - Coagulation factors X and IX contain O-glycosylations having N-acetylgalactosamine, which is a β-galactoside promoting their interaction with Cc-Lec. In addition, it has been reported that the glycosylations of the coagulation factors are important for their activity. The protein-protein docking study confirmed the obtained results by elucidating the modes of interaction and the types of linkages between Cc-Lec and factors X and IX through their Gla-domains, and calcium and magnesium ions.

Key words - Lectin, Docking protein-protein, Factors X/IX, Anticoagulation, Gla-domains.