EXPRESSION, PURIFICATION AND STRUCTURAL CHARACTERIZATION OF THE BRADYKININ-POTENTIATING PEPTIDE PRECURSOR PROTEIN

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The regulation of blood pressure is modulated by a number of active components including angiotensin II and bradykinin. The angiotensin-converting enzyme plays a pivotal role in the maintenance of the system producing angiotensin II and degrading bradykinin. The discovery of the first natural inhibitors for this enzyme, the bradykinin potentiating peptides (BPPs), led to the development of early drugs aimed at the control of unbalanced cardiovascular functions. The BPP precursor protein from Bothrops jararaca venom gland contains seven BPP sequences aligned tandemly, followed by a C-type natriuretic peptide at the C-terminus. Due to the unusual organization of these peptides in the precursor, the determination of the three-dimensional structure of this protein might reveal a novel protein fold and also provide insights into BPP activation. Therefore, the his-tagged fusion precursor protein was overexpressed in Escherichia coli Codon Plus strain and purified by employing immobilized-metal ion affinity chromatography. Far-UV circular dichroism spectrum showed a high content of α-helices (47%) and 18% of β-sheet and fluorescence data indicated that the ten tryptophan residues are buried in the tertiary structure shielded from solvent. Crystallization trials were performed by sitting-drop vapor diffusion method and the suitable crystals will be further submitted to x-ray diffraction experiments.

Key-words: Angiotensin-Converting Enzyme inhibitors, Blood Pressure Regulation, Bradykinin-Potentiation Peptide Precursor Protein, Protein Crystallization.

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