HETEROLOGOUS EXPRESSION AND REFOLDING OF ONE LECTIN FROM *BOTHROPS INSULARIS* SNAKE VENOM

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Bothrops insularis lectin (BiL) is a protein characterized as dimer of 30 kDa, composed of similar disulfide-linked monomers with calcium-dependent and saccharide-binding activity. In order to obtain recombinant BiL (rBiL), the cDNA coding region was amplified by PCR with specific synthesized oligonucleotides. The amplification product was inserted into plasmid pET14b to express rBiL in *E. coli* BL21 (DE3) cells. Among all tested conditions to induce expression the best was at 28ºC, with 0.1mM IPTG and lactose 20%. The rBiL was overexpressed as inclusion bodies and was solubilized and denatured in a urea-buffer. The presence of 16 kDa band was successfully confirmed by *Western Blot* using anti-His-tag antibody. After the nickel-affinity column, the rBiL was submitted to refolding by repeated dialysis rounds against the oxidation-buffer, with the presence of redox pair of cysteine-cystine, D-galactose and decreasing urea concentrations. The correct refolding of rBiL was confirmed by hemagglutinating assay. It was then applied in gel filtration column for separation of the homodimers. This experiment showed a major peak with retention time similar to native BiL (~ 18 ml). This protein is now available for structural and biological studies.

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