



Cloning of BIR2-domain from X-linked inhibitor of apoptosis protein in pET28a and its protein expression study

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ABSTRACT

Apoptosis is a cellular process of programmed death essential for homeostasis maintenance in multicellular organisms. Inappropriate apoptotic regulation has been implicated in many human diseases, including cancer and neurodegeneration. Inhibitors of apoptosis proteins (IAP) family of proteins, originally identified in baculoviruses, regulate programmed cell death in a variety of organisms, after caspase activation. The prototype member of the IAPs family, X-linked IAP (XIAP), contains three distinct baculovirus IAP repeat (BIR) domains and a C-terminal RING finger. The second BIR domain (BIR2) inhibits caspase-3 and -7, while the third BIR domain (BIR3) inhibits caspase-9. Inhibition of caspase-3 by XIAP is totally dependent on the interaction between the active site of caspase-3 and the linker region between the BIR1 and BIR2 domains of XIAP. In this research, the molecular cloning of XIAP-BIR2 domain was performed in pET28a vector. PCR product was generated using a standard PCR based cloning strategy. XIAP-BIR2 (amino acids 124–260) was amplified and ligated into pET28a plasmid between *NdeI* and *BamHI* restriction sites. Then the ligation mixture was transformed in the cloning host *Escherichia coli DH5α* and screened by antibiotic selection. Positive colonies were screened by colony PCR and double digestion of isolated plasmid and then sequenced to check the inserted DNA. After validation, plasmid containing BIR2 domain was transformed to *E.coli BL21 (DE3)* competent cells for optimization of recombinant protein overexpression. The aim of the present study is to determine drugs interaction with XIAP-BIR2 domain, in future.

Key words: Apoptosis, BIR Domain, IAPs Family, Cloning