Biosynthesis and interaction studies of apoptosis modulating proteins

Abstract

Industrial peptide production is commonly based on three alternative technologies: solid-phase synthesis, liquid-phase synthesis, and in vivo biotechnological recombinant technology. Chemical synthetic strategies are challenging in economic terms. Production of peptides with recombinant techniques proved to be a more effective. Peptides have received special attention in molecular biology in recent times, because peptides could be used in the study of protein structure and function. Apoptosis is an essential process in the development of cells. The inhibitor of apoptosis proteins suppresses cell death. This suppression is caused by inhibiting the activity of caspases. The mitochondrial protein SMAC (Second Mitochondria-derived Activator of Caspases) promotes apoptosis by eliminating the inhibitory effect of IAP's through physical interactions. The amino-terminal sequences in SMAC protein (AVPI) are required for this function. In the case of apoptosis researchers focused on small molecule Smac mimetics that target the *BIR3* domain IAP's. These IAP inhibitors are currently under investigation as anticancer drugs in clinical trials. In this work, I have studied biosynthesis of small peptides with 13 amino acid length. These peptides contain AVPI tetra peptide moiety of SMAC, which are involved in the interaction with IAP's. Furthermore, C-terminal labelling of peptides was executed by fluorescent labelling reaction. An Alexa Flour dye was used for crosslinking reaction. Interaction studies were performed by fluorescence polarization method. In addition, BIR3 domain of IAP's were also produced by recombinant techniques. During our research we investigated heterologous expression of IAP's. In this regard, solubilization from inclusion body and successful refolding of XIAP was executed using nondenaturing conditions. This research includes also in vitro investigation of protein-protein interaction by pull-down technique.