

Determination of the structure of the Mitochondrial associated human protein endozepine

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Apoptosis is a highly coordinated process important for the development and tissue homeostasis of multicellular organisms. Understanding this process is very important as deregulation of apoptosis leads to many pathologies such as cancer and autoimmune diseases. We are investigating the mechanism of action of endozepine in C2-ceramide induced apoptosis

Endozepine is a 10kDa protein described as a putative ligand of peripheral benzodiazepine receptor but its precise mechanism of action remains speculative. Endozepine belongs to the family of acyl CoA binding proteins which are thought to be important in fatty lipid synthesis and metabolism. Endozepine may be involved in the de-novo synthetic pathway of ceramide as a carrier protein or in the amplification of apoptosis inducing signals during mitochondria permeabilization. In order to understand the function of endozepine in apoptosis, this work was aimed at solving the structure of this protein in complex with ligands of interest.

¹⁵N- labeled Endozepine was over expressed in E.coli and purified using a GST purification system. ¹⁵N-HSQC NMR spectra were used to monitor titration binding interaction between endozepine, C2-ceramide and Palmitoyl CoA. These results showed that there was no binding interaction of endozepine to C2-ceramide but very strong binding to palmitoyl CoA. Endozepine has been labeled with ¹³C and ¹⁵N and this will be used for triple-resonance heteronuclear NMR experiments from which all protein backbone resonances will be assigned. This assignment would lead to the identification of residues involved in binding interaction thus providing basis for the pursuit of informative functional experiments.

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