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E.73 - Interaction Studies of the Human Hep1 Truncated Mutants and Negatively Charged Liposomes

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INTRODUCTION: The Hsp70 family of molecular chaperones are involved in several cellular proteostatis processes including protein folding/refolding and disaggregation, protein targeting for membrane traffic and degradation. Hsp70 have isoforms addressed to different cellular compartments. The human mitochondrial Hsp70 (HSPA9 or mortalin) has several functions, including its participation in the import of proteins from the cytosol into the mitochondrial matrix. To correctly perform their function, Hsp70 are regulated by its dynamic association with adenosine nucleotides and are assisted by co-chaperones. Hep1 (Hsp70-escort protein 1) is a co-chaperone responsible for maintaining HSPA9 in its soluble and functional state. Hsp70 also have the ability to interact with lipid membranes and the same was reported to hHep1. This co-chaperone is formed by a conserved zinc-finger domain core and poor conserved N- and C-terminals of unknown structure and function. OBJECTIVES: To elucidate the mechanism of interaction of hHep1 and N- and/or C-terminals truncated mutants with liposomes formed by cardiolipin and POPS. MATERIALS AND METHODS: The recombinant hHep1 truncated mutants were expressed in E. coli BL21DE3 strain by the pET28a expression vector and purified by Ni+2 affinity and size exclusion chromatographies. The stability of the mutants in solution and their interaction with POPS and Cardiolipin were investigated using techniques such as circular dichroism (CD) and Intrinsic Tryptophan Fluorescence. DISCUSSION AND RESULTS: The hHep1 mutants were obtained under suitable conditions to interact with POPS and Cardiolipin. In structural terms, the interaction of mutants with liposomes does not differ significantly from the interaction between liposomes and full-length hHep1. CONCLUSION: Deletion of the terminal regions of hHep1 does not significantly modify the interaction with POPS and Cardiolipin. The results obtained showed the importance of the hHep1 zinc finger domain present in the core region of the protein for the interaction with liposomes.

Keywords: Hep1, Liposomes, Molecular Chaperones

E.74 - Norclaurine synthase structural characterization using NMR

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INTRODUCTION: Norcoclaurine synthase (NCS) catalyzes the first step in the biosynthesis of benzylisoquinoline alkaloids (BIAs), a large and diverse group of natural products. Several BIAs are pharmacological active such as codeine, morphine and papaverine. Therefore, information about the mechanism of action of NCS is of great importance. NMR and crystallographic studies shows that NCS structure is similar to the Pathogenesis related-10 proteins. OBJECTIVES: Characterization of the protein dynamics, in its apo and holo form with both dopamine and (4-HPAA) for nuclear resonance magnetic (NMR) to better understand mechanism of catalysis. Also, we intend to compare the dynamics with proteins with similar fold such as: Bet v 1 and TTHA0849. MATERIALS AND METHODS: NCS was obtained by heterologous expression, and purification using ion exchange chromatography and gel filtration. Intrinsic fluorescence, circular dichroism and nuclear magnetic resonance were used for structure and dynamics characterization. DISCUSSION AND RESULTS: Initially, the protocol for protein expression and purification protocol was established. CONCLUSION: Once the protocol was established, at the current moment, dopamine titration experiments with the protein of NMR are being collected, so that can lead us to better information about the mechanism of action of NCS in its holo form, and also to compare the dynamics with proteins with similar fold.

Keywords: Nuclear Magnetic Resonance, Enzyme, Protein
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