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E.17 - Calcium interaction with HSPA1A Nucleotide Binding Domain

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INTRODUCTION: The HSPA1A protein belongs to the Hsp70 family of molecular chaperones, which are involved in the protein quality control by participating in folding of nascent proteins, protein delivery to traffic through membranes, protein aggregation prevention, protein disaggregation and refolding and targeting of client proteins to proteolysis, among others. It has two domains: Nucleotide Binding Domain (NBD) and Peptide Binding Domain connected by a hydrophobic linker, which are regulated by a reciprocal heterotrophic allosteric mechanism. Hsp70s can be related to several human pathologies, such as: neurodegenerative diseases, diabetes and some cancers. Because of that, it is important to know their regulation mechanisms, including the interaction with Ca^{2+} , an important cellular messenger. OBJECTIVES: Purification of the recombinant NBD construct of the HSPA1A and the evaluation of calcium and magnesium ions effects on the domain structure. MATERIALS AND METHODS: Recombinant hHSPA1A_NBD was produced in a bacterial system and purified by 2 chromatographic steps: Ni^{2+} affinity and size exclusion chromatographies. Then, the secondary and the local tertiary structures were evaluated by Circular Dichroism (CD) and Intrinsic Fluorescence emission, respectively. These techniques were also used to evaluate the effect of Ca^{2+} and Mg^{2+} in the hHSPA1A_NBD structure. DISCUSSION AND RESULTS: The biophysical evaluations demonstrated that hHSPA1A_NBD was obtained pure, in the monomeric and folded states. Intrinsic Tryptophan Fluorescence emission indicates that Ca^{2+} or Mg^{2+} caused quenching. In addition, while a blue shift was observed in the presence of Calcium, a subtle red shift was registered in the presence of Magnesium. Furthermore, the presence of Calcium ions caused a significant increase in the midpoint of a temperature transition curve, monitored by CD at 222 nm. CONCLUSION: hHspA1A_NBD was obtained folded and the presence of the divalent ions led to different conformation changes, which can indicate that they interact with different binding sites.
Keywords: HSPA1A, Calcium, Magnesium / **Supported by:** CNPq

E.18 - Biophysical Characterization of the Interaction Between Grb2 Protein and DNA G-quadruplex

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INTRODUCTION: Grb2 plays an important role in tyrosine kinase-mediated signal transduction, including binding of receptor tyrosine kinases to the Ras/MAPK pathway, which is implicated in oncogenic outcome. Due to its importance within the cell, is a potential target of studies to test and evaluate its interactions, such as the case of DNA G-quadruplex, composed of a guanine rich sequence and is present in DNA regions with high biological significance. Studies have described the presence of G-quadruplex in oncogene promoters, telomeres, and transcription start sites. The presence of these structures in such specific locations confirms that they play a crucial role in controlling a variety of cellular processes. OBJECTIVES: Considering the importance of DNA G-quadruplex, this work aimed to study its interaction with the Grb2 protein by fluorescence. MATERIALS AND METHODS: Grb2 was expressed in E. coli: BL21(DE3). It was realized an affinity purification with cobalt-affinity column IMAC-HiTrap-HP in buffer 500mM imidazole, 50mM Tris-HCl (pH 8.0), 100mM NaCl and 1mM β-ME. After, was applied in Sephacryl-100 resin in buffer 20mM Na_2HPO_4/NaH_2PO_4 (pH 7.0), 50mM NaCl and 1mM β-ME for size-exclusion chromatography. The binding of DNA to Grb2 was analyzed by fluorescence spectroscopy at temperatures of 288, 298 and 308K, in the presence and absence of DNA. DISCUSSION AND RESULTS: In a preliminary analysis, our results show that DNA interacts with Grb2, evidenced by fluorescence quenching of Grb2 as a function of increasing ligand concentration. Thermodynamic analysis of this interaction reveals to us a ΔH, $\Delta S < 0$ characteristic of Hydrogen bond or Van der Waals type interactions. Also, it was found that the protein-ligand binding is of the 1:1 type, that is, one ligand for each protein. CONCLUSION: This study allows us to understand at a molecular level the microenvironment in which Protein-DNA interacts and how it can be physically and structurally affected.
Keywords: DNA G-quadruplex, Fluorescence Spectroscopy, Grb2 / **Supported by:** FAPESP/Cnpq