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E.33 - Studies on mitochondrial chaperone system proteins TRAP-1 and CyP-D, characterization e interaction

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INTRODUCTION: Many of the biological functions are performed by proteins, including the maintenance of cellular homeostasis. Molecular chaperones are essential in this process, such as mitochondrial HSP90, TRAP-1, due to their ability to ensure correct protein folding and protect the cell from apoptosis. TRAP-1 is responsible for maintaining mitochondrial integrity and playing a protective role against reactive oxygen species. In this context, Cyclophilin-D (CyP-D) is also highlighted, a co-chaperone that regulates the activities of other interacting proteins. Both proteins are heavily studied as therapeutic targets, as imbalances in their functionality are related to diseases such as cancer. OBJECTIVES: The production of proteins was the initial objective, followed by the main focus of characterizing both recombinant proteins, as well as detecting and evaluating the *In vitro* interaction between them. MATERIALS AND METHODS: Both proteins were produced in E. coli BL21(DE3), and protein expression was induced by IPTG. Purification was achieved through immobilized metal affinity and size-exclusion chromatographies. The efficiency of the methods was verified by SDS-PAGE and protein concentration was determined by spectrophotometric measurements. A pulldown assay was performed to detect the interaction, and both proteins were submitted to analytical gel filtration for characterization. DISCUSSION AND RESULTS: Trap-1 and Cyp-d were efficiently expressed and purified, soluble, with a high degree of purity and homogeneity. Both were well characterized by analytical gel filtration and the pulldown assay was able to detect the interaction between Trap-1 and Cyp-d. CONCLUSION: Proteins were successfully produced, applying selected methodologies and protocols. TRAP-1 was detected as an asymmetric dimer in solution, while CyP-D was found as a globular monomer. The pulldown assay confirmed the physical interaction between the proteins under all conditions tested, which justifies and encourages a more in-depth and detailed investigation of this interaction, in the way of the search for the thermodynamic signature of the interaction and involvement in the mitochondrial chaperone system.

Keywords: Molecular chaperones, Trap-1, Cyp-D / **Supported by:** CNPq and FAPESP

E.34 - DNA-mediated Fabrication of Bradykinin Nanostructures

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INTRODUCTION: Introduction: Bradykinin (BK) is a short peptide-hormone belonging to the kinin-kallikrein system, having a vasodilator effect, and being related to the release of inflammatory mediators. For this reason, it has been explored as a blood pressure reducer, and its application is well established in the pharmaceutical industry as an active ingredient for antihypertensive drugs. On the other hand, no reports are available on nanostructured BK materials. An important BK analog is des-Arg9-BK (DBK), whose sequence is almost identical to that of BK, but lacking an arginine residue. DBK has a different mechanism of action, being an agonist of the B1 receptor. OBJECTIVES: Objective: We aimed at producing supramolecular assemblies based on BK and DBK. We also intended to provide detailed structural data on these assemblies and investigate if they exert cellular responses similar to the peptide in its monomeric form. MATERIALS AND METHODS: Methods: BK and DBK were complexed with DNA fragments. Steady-state fluorimetry was used to determine critical aggregation concentrations, whereas the secondary structure was analyzed through circular dichroism experiments. Atomic force microscopy and small-angle X-ray scattering were used to investigate the nanoscopic structure of the complexes. The cellular response was evaluated by monitoring calcium influx in HuVEC cells. DISCUSSION AND RESULTS: Results: We have found that DNA behaves as a template for BK strands, leading to the formation of nanoscopic fibrils stabilized by β-sheets. Interestingly, the same effect is not observed for DBK. We also identified that BK preferentially binds to the major grooves of DNA duplexes, whereas DBK intercalates in-between nucleotide bases. Importantly, BK-DNA fibrils were found to modulate calcium influx in HuVEC cells, thus preserving the bioactivity of the native peptide. CONCLUSION: Conclusion: We demonstrated a viable strategy to fabricate nanostructured matrices based on BK, which are potentially exploitable for the development of biomaterials endowed with hypotensive capabilities.

Keywords: peptide-hormone, DNA-peptide complexes, nanostructure / **Supported by:** FAPESP