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E.55 - Modulation of Alpha-synuclein Aggregation by Organic compounds

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INTRODUCTION: Parkinson's disease (PD) is the second most prevalent neurological disorder and is characterized by the degeneration of dopaminergic neurons in the substantia nigra and the subsequent loss of dopamine innervation on striatum. Affected neurons accumulate intracytoplasmic inclusions called Lewy bodies, which are composed mainly of α-synuclein (α-syn) protein. α-syn aggregates are also associated to other neurodegenerative disorders known as synucleinopathies. Several lines of evidence suggest that small aggregates such as oligomers are the most toxic forms of α-syn. Thus, compounds that reduce the formation of toxic α-syn species may be of interest to PD therapy. OBJECTIVES: Considering that α-syn has prion-like properties, we aimed to investigate the efficacy of trimethoxy-chalcones (J8, LC90 and LC91) previously shown to have anti-prion activity, in modulating the aggregation of wild-type and mutant forms of α-syn. LC90 and LC91 were previously shown to remodel α-syn fibrils. MATERIALS AND METHODS: We expressed and purified recombinant α-syn (WT and the E46K mutant) to produce oligomeric species of α-syn. Aggregation was monitored by binding of thioflavin-T. Aggregated α-syn were characterized by Dynamic Light Scattering (DLS) and Transmission Electron Microscopy (TEM). DISCUSSION AND RESULTS: Our preliminary TEM results showed that we obtained both fibrils and oligomeric α-syn species after the aggregation protocol, with a wide hydrodynamic radious range, as evidenced by DLS. Aggregation in the presence of J8 resulted in more organized α-syn fibrils, indicating fibril eemodeling by this trimethoxy-chalcone. CONCLUSION: We will next evaluate the *In vitro* and *In vivo* efficacy of the selected organic compounds in mammalian cell culture and in animal models of PD.
Keywords: Aggregation, Alpha-synuclein, Oligomers / Supported by: CNPq

E.56 - Investigating the molecular interaction of human Bip (Heat Shock Protein A5) with Calcium

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INTRODUCTION: 70 kDa Heat shock proteins (Hsp70) are ubiquitous molecular chaperones acting in protein homeostasis, including folding of nascent proteins, translocation of polypeptides through membranes, assembly and disassembly of protein complexes, targeting proteins for clearance, among other functions. HSPA5, also called immunoglobulin binding protein (BiP), is the human Hsp70 abundantly present in the endoplasmic reticulum (ER). Under stressful conditions, HSPA5 migrates to the nucleus, cytoplasm, mitochondria and cell surface, in which it performs functions related to cell proliferation, apoptosis, regulation of innate and adaptive immunity and modulation of the response to unfolded protein response. Recently, it has been seen that the Ca^{2+} strengthening the Hsp70 binding to the adenosine nucleotides, mainly to the ADP. Such interaction would lead Hsp70 to a slower releasing of client proteins, letting a longer time of chaperone-client interaction, which should allow to reach an adequate client-protein fate. OBJECTIVES: To study the molecular interaction of the recombinant HSPA5 (rHSPA5) with calcium ions. MATERIALS AND METHODS: rHSPA5 was produced in bacterial system and purified by 2 chromatographic steps: Ni $^{2+}$ affinity and size exclusion. The interaction with Ca^{2+} was monitored by circular dichroism (CD), intrinsic tryptophan fluorescence emission, isothermal titration calorimetry (ITC), differential scanning calorimetry (DSC) and ATPase activity. These experiments were done in comparison to Mg^{2+}. DISCUSSION AND RESULTS: Our results have shown that rHSPA5 was obtained in the folded and functional states. The presence of Ca^{2+} led to a reduction of the ATP hydrolysis rate in comparison to the presence of Mg^{2+}. The intrinsic fluorescence of tryptophan showed that calcium causes a suppression in the protein fluorescence, while ITC and DSC have shown some differences regarding its interaction with nucleotides in comparison with assays in the presence of Mg^{2+} CONCLUSION: These results help in a better understanding and guiding future studies on the Hsp70 chaperone family.
Keywords: HSPA5, Calcium, Molecular chaperone
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