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E – Structural Biology

E.01 - Production of the Human Hsp70-Escort Protein (hHep1) for Structural Analysis by NMR

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INTRODUCTION: Heat shock proteins (HSP) are molecular chaperones involved in several processes in the protein homeostasis. An important family of heat shock proteins is the Hsp70, that participate in the aggregation prevention, protein disaggregation, protein targeting for clearance and protein folding/refolding, among others. However, several co-chaperones assist Hsp70 to perform such multitude of functions. One of them is hHep1 (human Hsp70-escort protein 1) that stimulates its ATPase activity and prevents the aggregation of human mitochondrial Hsp70 (mtHsp70 or HSPA9) as well as client proteins. The structural study of this human co-chaperone is important to gain further insights about how it regulates mtHsp70 and help in the functions of the later protein. hHep1 has a zinc-finger domain and poorly folded N- and C-terminal regions of unknown functions. The yeast Hep1 (yHep1) is the best-known orthologue, but yHep1 and hHep1 are 30% identical and have some divergent properties and are not functionally equivalent. OBJECTIVES: Express hHep1 in M9 minimal medium to purify it in the folded state for NMR structural characterization. MATERIALS AND METHODS: The pQE2::hHep1 expression vector was transformed in *Escherichia coli* BL21 (DE3) cells and induced in M9 minimal medium. The expressed hHep1 was purified by Ni $^{2+}$ -affinity and preparative size exclusion chromatographies. Such protein was characterized by circular dichroism, intrinsic fluorescence spectroscopy and SDS-PAGE. DISCUSSION AND RESULTS: hHep1 was satisfactorily expressed in M9 minimal medium which allowed to purify it as monomer and to be obtained in high concentrations (around 600 µM of hHep1). Its spectroscopy signatures were confirmed by the biophysical tools. CONCLUSION: hHep1 protein was successfully obtained in minimal medium with a good yield. This result opens the possibility to it be structural characterized by NMR
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E.02 - Development of Nanostructured Matrices Based on Antidiabetic Peptides

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INTRODUCTION: Diabetes has high prevalence in Brazil and all around the world. The number of people with diabetes is expected to be near to 366 million by 2030. Therefore, the search for anti-glycemic agents is a relevant matter with a potential impact in the public health. The development of therapies based on nanostructures is at the frontline of nanomedicine, representing a revolutionary way to treat different health conditions. Our objective was to develop nanostructured materials with anti-glycemic properties. The production of nanostructures was based on liraglutide, a peptide with 31 residues, and desmopressin, an ultrashort cyclic peptide. These compounds are available in the pharmaceutic market, and they are currently used to treat diabetes mellitus and diabetes insipidus, respectively. OBJECTIVES: To develop nanoparticles based on anti-glycemic peptides and provide information about their structure, from the molecular level to the nanometer range. MATERIALS AND METHODS: The structural analyses were performed through spectroscopic techniques (UV-Vis, fluorimetry and circular dichroism), whereas morphology assays were carried out using atomic force microscopy. DISCUSSION AND RESULTS: Both liraglutide and desmopressin were found to form nanometric aggregates at critical concentrations near to 0.5 mg/ml, with b-sheet conformations dominating the secondary structure of the aggregates. Quenching of tryptophan (or tyrosine) emission indicated a major role of hydrophobic domains in the self-assembly. The interaction of peptides with lipid membranes was also demonstrated, showing that their secondary structure is modified in the presence of membranes, and interaction with lipid bilayers being driven by hydrophobic domains. CONCLUSION: We demonstrated the fabrication of ordered nanoassemblies based on liraglutide and desmopressin, which are stabilized by b-sheets. It was also verified that these nanoparticles interact with lipid membranes, suggesting they can associate to cell membranes. To our knowledge this is the first study reporting the production of nanostructured materials from peptides endowed with anti-glycemic activity.
Keywords: Diabetes, Liraglutide, Desmopressin