F.35 - Optimization of the Process of Expression in E. coli and Purification of the Catalytic Sites of the ACE1 by the ELP-Intein System

Beatriz Angelo Prata[1], Carolina Machado dos Santos[1], **Regina Affonso [1]**
[1]Centro de Biotecnologia, Instituto de Pesquisas Energéticas e Nucleares (São Paulo, Brasil)

INTRODUCTION: Angiotensin I-converting enzyme (ACE) is a fundamental part of the renin-angiotensin system; this has two domains, N- and C-, each of which has a catalytic site that exhibits 60% sequence identity. Its actions are in the control of blood pressure, protection of the brain by cleavage of beta-amyloid bodies, cell proliferation, formation of hematopoietic stem cells, among others. OBJECTIVES: Obtaining the catalytic sites Ala361 to Gly468 (N domain region, csACEN) and Ala959 to Ser1066 (C domain region, csACEC) in pure form and with their correct structural conformation. MATERIALS AND METHODS: Expression conditions of pE1csACEN and pE1csACEC vectors in E. coli BL21(DE3) strain: cultures grown in Terrific Broth at 37ºC at 140 rpm for 20–24 h and 0.1 mM IPTG. Purification by Elastin-like Polypeptide (ELP) precipitation: ELP-bound catalytic sites were purified with two ammonium sulfate precipitations (ASp). Remotion of ELP: by autocleavage of the Intein sequence using the buffers: sodium phosphate, sodium cacodylate, MES and Tris-HCl. The ELP/Intein was removed from the sample by ASp. The analyzes of all stages of the process were performed by SDS-PAGE and Dot blotting. DISCUSSION AND RESULTS: The differential for obtaining the pure peptides was the temperature of 37ºC, with a significant increase in expression concerning the cultivation of 16ºC. In the ELP purification steps, ammonium sulfate buffer concentrations of 0.57 M and 0.8 M were the most efficient. Intein's self-cleaving was more efficient with MES buffers and Tris-HCl for ELPsACEN and ELPsACEC, respectively. Structural analysis by Circular Dichroism and Fluorescence confirmed the correct structure of the pure peptides. CONCLUSION: In the present work, we defined the most efficient conditions for expression, purification, and obtaining of ACE catalytic sites in pure form. The csACEN and csACEC peptides will allow greater assertiveness in obtaining and characterizing new hypertensive drugs and in the hydrolysis of substrates such as beta-amyloid.

Keywords: Catalytic sites of ACE, ELP/ Intein, high temperature of expression

F.36 - Biophysical and Structural Studies of a Novel Und-type Decarboxylase Active in Fatty Acids

Raul De Castro Cunha Claudino [1], Letícia Leandro Rade[1], Mayara Chagas de Ávila[1], Gabriela Felix Persinoti[1], Wesley Cardoso Generoso[1], Ricardo Rodrigues de Melo[1], Letícia Maria Zanphorlin[1]
[1]Brazilian Biorenewables National Laboratory, Brazilian Center for Research in Energy and Materials (SP, Brazil)

INTRODUCTION: Alkenes are an important platform for producing chemical building blocks and drop-in biofuels. After the discovery of the Und decarboxylase from *Pseudomonas sp*. that decarboxylates fatty acid into 1-undecene, the scientific and technological interest in this family of enzymes vastly increased. OBJECTIVES: Our research focuses on finding new Und-type decarboxylases with fatty acid decarboxylation activity to understand what drives that reaction and propose a biotechnology route to produce alkenes. MATERIALS AND METHODS: Herein, we explored a microbial consortium in the presence of different sources of fatty acids to identify a novel Und-type decarboxylase. The selected enzyme was expressed in a host cell (*E. coli*) and purified by chromatography methods. The purified enzyme was submitted to biophysical studies, crystallization assays and X-ray diffraction experiments to determine its molecular properties. DISCUSSION AND RESULTS: The results revealed that regardless of the source of fatty acid used, the predominance of microorganisms belonging to the phylum *Proteobacteria* was highlighted. The enzyme was purified in a folded form, presenting a Tm of 53.7 ºC. The elucidation of the tertiary structure shows the fatty acid molecule in the binding pocket oriented by the conserved catalytic residues (Glu103, His106, and His196). CONCLUSION: Taken together, these results will help us understand the relationship between the structure and function of these enzymes and their molecular mechanisms. Ultimately, this project has enormous potential to contribute to developing biological routes to produce drop-in biofuels and other bioproducts derived from terminal alkenes.

Keywords: *Alkenes, Decarboxylase, Drop-in biofuels*
Supported by: *FAPESP*