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D.33- The N and C-Terminus Is Essential for Hsp70-Escort Protein 1 Function and Cell Membrane Interaction

Moritz, M. N. O.1, Gomes, M.J.M.1,1, Oliveira, S.D.1,1, Silva, N.S.M.1,1, Leitão, A.1,1, Borges, J.C.1,1
1Sao Carlos Institute of Chemistry, University of Sao Paulo (SP, Brasil)

Chaperones are specialized in protein folding and cooperate during protein quality control in eukaryotic cells. Human 70 kDa heat shock proteins (Hsp70 or HSPA) comprise a highly conserved chaperone family with a pivotal role in cellular homeostasis. However, mitochondrial Hsp70 (like HSPA9) themselves are prone to aggregate and need for a special co-chaperone called Hsp70-escort protein 1 (Hep1). Human Hep1 (hHep1) is capable of maintaining recombinant HSPA9 soluble and to stimulate its ATPase activity. Additionally, hHep1 remodels HSPAs supramolecular assemblies (SMAs) into small particles and has intrinsic chaperone activity. Recently, we demonstrated that hHep1 interacts with liposomes and cell membranes. The hHep1 core region, housing a zinc finger domain, is pivotal for its activity regarding HSPA9. However, the significance of the C and N-terminus regions for hHep1 functions remains unexplored. This study aimed to investigate three truncated forms of hHep1: Ndel (N-terminus deleted), Cdel (C-terminus deleted) and Core region (both N- and C-terminus deleted) in order to understand which region is responsible for its interaction with cell membrane. We performed cellular incorporation assays with recombinant hHep1 truncated constructions and analyzed them by western blotting. Membrane filter retention assays with HSPAs SMAs were done to demonstrate if hHep1 truncations disassembles the SMAs. Intrinsic chaperone activity was assessed by light scattering profile. We observed that the hHep1 core construction was less incorporated by the cells when compared to Cdel, Ndel and the full-length hHep1. These results suggest that the N and C-terminus are essential for cell membrane interaction. We also demonstrated that the terminal ends of hHep1 are important to dissemble HSPAs thermal SMAs. Ours results suggest that N and C-terminus regions have critical roles in the intrinsic chaperone activity of hHep1 as well as cell membranes interaction. Keywords: Chaperones, Cochaperones, HSPAs

D.34- Catalytic and Structural properties of Fe(II)-dependent fatty acid decarboxylases for green chemical products

Melo, V. S.1,2, Avila, M.C.1,2, Rade, L.L.1, Melo, R.R.1, Miyamoto, R.Y.1, Generoso, W.C.1, Zanphorlin, L.M.1
1Laboratório Nacional de Biorrenováveis, Centro Nacional de Pesquisa em Energia e Materiais (SP, Brasil),
2Faculdade de Engenharia de Alimentos, Universidade Estadual de Campinas (SP, Brasil)

The global transition aiming at a sustainable energy matrix for minimizing dependence on fossil fuels has led to a noticeable interest in biohydrocarbons production. These stand out as viable alternatives due to their direct compatibility with existing infrastructure and chemical similarity to petroleum derivatives. Among the promising strategies is the enzymatic decarboxylation of fatty acids, catalyzed by non-heme iron-dependent oxidases, such as UndAs, capable of converting fatty acids into n-alkenes without the need for cofactors. This study aims to identify and elucidate the mechanisms of action of type UndA decarboxylases since they perform non-conventional oxidative reactions. We selected UndA enzymes by sequence similarity network, which were expressed in E. coli BL21 (DE3) and purified using affinity and size-exclusion chromatography. The ability to produce n-alkenes from fatty acids (C8 to C16) was evaluated using gas chromatography and mass spectrometry. We also studied the impact of variables such as pH, temperature, and ascorbic acid concentration on catalytic activity. Crystallization assays were conducted under various conditions and the crystals obtained were diffracted. UndA828 and UndA676 emerged as the most effective, generating high quantities of alkenes from the tested fatty acids. These enzymes showed greater activity under mild temperature conditions and slightly acidic pH, as well as in reduced concentrations of ascorbic acid. The crystalline structure of UndA828 was determined, revealing an active site containing an iron atom and essential conserved amino acids for its functionality. Based on structural data, mutants have been designed to better understand the specificity and (regio) chemoselectivity. The findings might provide valuable insights into the enzymatic decarboxylation of fatty acids, an atypical reaction, by non-heme oxidative enzymes. Moreover, the discovery and development of potential fatty acid decarboxylases significantly contributes to the biotechnological approaches towards the sustainable production of olefins.
Keywords: Decarboxylases, Metalloenzymes, UndA