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D.47- Biochemical and Structural Evaluation of the Recombinant HSPA5 Supramolecular Assemblies

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The HSP70s represent an important molecular chaperones family playing diverse critical roles in protein homeostasis. Within this family, the HSPA5, which is also known as BiP (Binding immunoglobulin Protein), stands out the most abundantly expressed and uniquely found form in the endoplasmic reticulum (ER) lumen. HSPA5 can be also found in mitochondria, cytoplasm, nucleoplasm, cell surface, and exosomes. Structurally, it consists of two domains connected by a hydrophobic linker: the nucleotide-binding domain (NBD) and the substrate-binding domain (SBD). Although predominantly monomeric, HSPA5 also has the ability to oligomerize, forming supramolecular assemblies (SMAs), a process associated with intermolecular disulfide bridge interactions involving cysteine residues in the NBD (Cys41) and SBD (Cys420). The ER is vital for lipid synthesis, post-translational modifications, and protein folding of newly synthesized proteins, favoring disulfide bond formation depending on the redox environment. Therefore, the ER-resident Hsp70 facilitates protein folding, and it has been shown that its activity is affected by the redox environment. Our purpose is investigate how the redox environment modulates HSPA5s SMAs via intermolecular disulfide bridges. The recombinant HSPA5 was expressed, purified, and subjected to heating (42 ºC, 2 h) to induce SMAs formation. Once formed, they were re-purified by size-exclusion chromatography to obtain them free from the monomeric fraction. Additionally, biophysical analyses regarding the secondary, tertiary, and quaternary structure of SMAs, as well as ATPase activity assays to assess their functionality. Recombinant HSPA5 was produced, and its SMA was obtained through a thermal treatment partially folded and showing partial ATPase activity. Treatment of the SMA with monomeric HSPA5 (not subjected to thermal treatment) indicates that, in the presence of a reducing environment, monomeric HSPA5 leaded to the SMAs disassembly. In contrast, in an oxidizing environment, the monomeric form caused SMAs degradation. The action of the monomeric HSPA5 on promoting SMAs disassembly is dependent on the redox environment. These findings highlight the sensitivity of HSPA5 function to redox variations in the ER, implying a critical role of these changes in modulating protein folding and cellular homeostasis. Keywords: Protein aggregation, Redox Environment, HSPA5

D.48- Structure-function relationships defining substrate specificity of short-chain dehydrogenase/reductases (sdr) active on monolignols

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Metabolic pathways have been described for the catabolism of lignin-derived aromatics, a potential renewable carbon source alternative to petroleum. However, considering the main lignin precursors the monolignols, p-coumaryl, coniferyl, and sinapyl alcohols - only coniferyl alcohol oxidation by the CalAHR199 enzyme has been reported so far. CalAHR199, a short-chain dehydrogenase/reductase (SDR) family member, lacks clarity regarding its substrate range and the underlying structure-function relationships driving its activity on monolignols. To broaden the enzyme portfolio for monolignols oxidation and address these knowledge gaps, our work identified and characterized MolA, an aryl-alcohol dehydrogenase, sharing 60% sequence identity with CalAHR199. We investigated MolA's substrate profile, kinetics, and structure-function relationships using biochemical assays and X-ray crystallography, comparing with CalAHR199s substrate profile and structure prediction. Our results reveal that MolA and CalAHR199 exhibit significant activity on all three monolignols, with minimal activity on benzyl alcohol derivatives. Kinetic assays on MolA demonstrated its two orders of magnitude greater efficiency (Kcat/Km) on monolignols compared to vanillyl alcohol. Structural analyses suggests that hydrophobic interactions stabilize the aromatic ring of the substrate in a subsite that allows the alcohol group of monolignols, but not of benzyl alcohol derivatives, to make hydrogen bonds with the catalytic residues Ser117 and Tyr157, explaining our findings. Interestingly, CalAHR199 was more active on coniferyl alcohol, while MolA showed higher specific activity over p-coumaryl alcohol. Probably, a Thr208 residue in CalAHR199 stabilizes the coniferyl alcohol by hydrogen bonding with the oxygen of the 3-methoxy group. In MolA, the hydrophobicity of Val208 may favor more stable interactions with the aromatic ring of p-coumaryl alcohol, contributing to MolA preference for p-coumaryl alcohol over coniferyl alcohol. In summary, this study presents the first evidence of bacterial SDRs converting all three primary monolignols into aldehydes, revealing a novel SDR member and uncovering structure-function relationships of these enzymes. Keywords: structural biology, aryl-alcohol dehydrogenases, substrate specificity