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XXII NATIONAL CONGRESS OF BIOCHEMISTRY
Next-Gen Biochemical Challenges Across Borders

PC2.1: Mitochondrial glutamyl-tRNA synthetase and its role on Leukoencephalopathy – a structural and biochemical characterization of disease variants

Andreia A. Dias; Joana V. Ribeiro; Cláudio M. Gomes; Bárbara J. Henriques

BioISI – Biosystems and Integrative Sciences Institute and Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal.

Corresponding author:
Andreia A. Dias; fc54368@alunos.fc.ul.pt
Joana V. Ribeiro; mjvribeiro@ciencias.ulisboa.pt
Cláudio M. Gomes; cmgomes@ciencias.ulisboa.pt
Bárbara J. Henriques; bjhenriques@ciencias.ulisboa.pt

Background: Mitochondrial aminoacyl-tRNA synthetases (mt-aaRS) are proteins responsible for adding an amino acid to the corresponding tRNA and, consequently, they are indispensable for the translation process in mitochondria. Interestingly, an increasing number of protein variants are being identified, including in Portugal, associated to specific mitochondrial disorders (MD). Hence, it is essential to clarify the molecular mechanisms behind these MDs and to provide information on mt-aaRS structure, conformation, and function.

Aim(s): We aim to study mitochondrial glutamyl-tRNA synthetase (EARS2) wild-type and three disease variants to uncover possible genotype-phenotype correlations.

Methods: Proteins were heterologously expressed in *E.coli* and purified using chromatographic techniques. The pure proteins were characterized regarding structure and stability using techniques such as circular dichroism (CD), fluorescence spectroscopy and differential scanning fluorimetry.

Results: Disease variants express at lower yields than wild-type protein. The EARS2-p.Glu96Lys variant was co-expressed with molecular chaperone GroEL/GroES enabling it's production. Purified EARS2-WT and EARS2-p.Glu96Lys present an identical folded conformation distinguished by far-UV CD. However, the variant presents lower thermal stability, with an apparent melting temperature 4°C lower than wild-type.

Conclusions: We believe that implementing the purification protocol for this mt-aaRS will open new avenues for characterizing variants and, in the future, aid in designing disease therapies.

Keywords: Mitochondrial aminoacyl-tRNA synthetases, Structural Biochemistry, Protein conformation and stability.

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PC2.10The impact of succinylation on Medium Chain Acyl-CoA Dehydrogenase (MCAD) is regulated by Sirtuin5

Domingos, R.; Ribeiro, J. V.; Henriques, B. J.*

BioISI – Biosystems and Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal, and Departamento de Química e Bioquímica, Faculdade de Ciências,Universidade de Lisboa, Lisboa, Portugal.

Corresponding author: Ruy Domingos; ruymdomingos@gmail.com

Metabolic regulation involves a complex interplay of genomic, proteomic and metabolic adjustments within cells. A particular group of non-enzymatic post-translational modification (PTMs) known as acylations, such as succinylation and glutarylation, have emerged as important regulators of mitochondrial enzymes via sirtuin-mediated regulatory pathways [1]. The extent of acylations is closely associated with the accumulation of intermediate metabolites that occur under certain conditions such as caloric restriction and in several metabolic disorders [2]. In fact, we have shown that glutaryl-CoA dehydrogenase is prone to high levels of glutarylation, due to increased glutaryl-CoA production stimulated by lysine catabolism, which diminishes enzyme activity and is regulated by sirtuin5 (Sirt5)[3].

Here we aim to evaluate acylation effects on MCAD enzyme structure and function using a combination of biochemical and biophysical methods.

Our results show that purified MCAD is easily succinylated and glutarylated. Through spectroscopic techniques such as circular dichroism or fluorescence we were able to demonstrate that succinylation has no major implication on MCAD's structure. Further, we access protein thermal stability and observe no major changes due to succinylation. Nonetheless, this modification induces an increase of enzymatic activity. In addition, we show that Sirt5 reverts succinylation and brings function to similar levels to unmodified MCAD.

Keywords: Acylation; Flavoprotein; Structural Biochemistry; Biophysical methods; Enzymatic Activity.

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PC8.1: Developing new Biological Adhesives inspired by Sea Urchin proteins

Mariana R. Santos [1], Cláudio M. Gomes [1], Romana Santos [2], Bárbara J. Henriques [1]

[1] BioISI – Biosystems and Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, and Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal.

[2] MARE – Marine and Environmental Sciences Centre, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal, and Departamento de Biologia Animal, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal.

Corresponding author: Mariana R. Santos; mrosantos@ciencias.ulisboa.pt

Background: There is a significant demand for biological adhesives that are non-cytotoxic and efficient in wet/humid environments for biomedicine/biotechnology applications (i.e., surgical adhesives). Marine invertebrates produce secretions with remarkable adhesive properties in seawater, which closely resembles physiological fluids, and can inspire the development of new biomimetic adhesives. In our research, we have focused in studying sea urchins' adhesives, having identified Nectin from *Paracentrotus lividus* as an important adhesive protein present in its adhesive organs and secretions [1].

Aim(s): Our current objective is to develop a novel bioadhesive inspired in sea urchins' adhesive proteins. To accomplish this, we are producing and characterizing constructs inspired in the Nectin protein.

Methods: We resort to recombinant protein production, and a variety of techniques such as circular dichroism (CD), fluorescence, transmission electron microscopy (TEM) and surface coating assays to characterize the purified constructs in respect to structure, conformation, aggregation propensity, and adhesive strength.

Results: We have successfully produced one construct that, in the presence of specific salts at high concentrations, tends to self-assemble, as reported by surface coating assays, Thioflavin-T fluorescence binding assays and TEM imaging evidencing possible fibre formation.

Conclusions: Our findings suggest a promising path toward the development of a new bioadhesive material for biomedical and biotechnological applications.

Keywords: Marine bioadhesives, nature-based solutions, recombinant proteins, biophysical methods.

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