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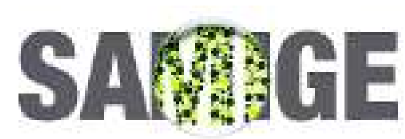

LVI SAIB Meeting – XV SAMIGE Meeting

SAIB-SAMIGE Joint Meeting 2020 – *Online*

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Cover image:

Mineral–microorganisms interactions

Mlewski EC[1], Gérard E[2]
[1]Centro de Investigaciones en Ciencias de la Tierra CICTERRA-CONICET-UNC. [2]Institut de Physique du Globe de Paris, IPGP.

A Confocal Laser Scanning Microscopy image of a resin-embedded microbialite from Laguna Negra (Puna-Catamarca), stained with calcein (a fluorescent dye that produces a stable complex in the presence of calcium and fluoresces in the green region of visible light). Mineral aggregates are observed in blue. Their surfaces are partially stained with calcein, indicate the presence of free Ca^{2+} ions. Diatoms and *Rivularia halophila* filaments are visible in red thanks to their photosynthetic pigments.

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Argentine Society for Biochemistry and Molecular Biology
(SAIB)

XV Annual Meeting
Argentinean Society for General Microbiology
(SAMIGE)

SAIB-SAMIGE – Online
Joint Meeting 2020

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ENZIMOLOGY

EN-C01-98
CHARACTERIZATION OF SdGA, A COLD-ADAPTED GLUCOAMYLASE FROM *Saccharophagus degradans*

Wayllace NM, Hedin N, Busi MV, Gomez Casati DF
Centro de Estudios Fotosintéticos y Bioquímicos (CEFOBI-CONICET) – Facultad de Ciencias Bioquímicas y Farmacéuticas (UNR). E-mail: wayllace@cefobi-conicet.gov.ar

We investigated the structural and functional properties of SdGA, a glucoamylase (GA) from *Saccharophagus degradans*, a marine bacterium which degrades different complex polysaccharides athigh rate. SdGA is composed mainly by a N-terminal GH15_N domain linked to a C-terminal catalyticdomain (CD) found in the GH15 family of glycosylhydrolases with an overall structure similar to otherbacterial GAs. The protein was expressed in *Escherichia coli* cells, purified and its biochemical properties were investigated. Although SdGA has a maximum activity at 39°C and pH 6.0, it also shows high activity in a wide range, from low to mild temperatures, like cold-adapted enzymes. Furthermore, SdGA has a higher content of flexible residues and a larger CD due to various amino acid insertions compared to other thermostable GAs. We propose that this novel SdGA, is a cold-adapted enzyme that might be suitable for use in different industrial processes that require enzymes which act at low or medium temperatures.

EN-C02-102
UNDERSTANDING CARBON METABOLISM IN GREEN ALGAE: CHARACTERIZATION OF *Chlamydomonas reinhardtii* PEPCK

Torresi F, Gomez-Casati D, Martín M
Centro de Estudios Fotosintéticos y Bioquímicos (CEFOBI- CONICET), Facultad de Ciencias Bioquímicas y Farmacéuticas (UNR). Email: torresi@cefobi-conicet.gov.ar

Phosphoenolpyruvate carboxykinase EC 4.1.1.32 (GTP-dependent) or EC 4.1.1.49 (ATP-dependent) is a widely distributed enzyme which catalyses the reversible decarboxylation and phosphorylation of oxaloacetate (OAA) to yield phosphoenolpyruvate (PEP) and carbon dioxide (CO2), using ATP or GTP for the phosphoryl transfer, and requires a divalent metal ion for activity. PEPCKs can be divided into two groups, based on its strict specificity towards the nucleotide substrate: the ATP-dependent family, found in bacteria, yeast, higher plants and trypanosomatids; and the GTP-dependent family, found in molluscs, fungi, insects, and vertebrates. The primary role of PEPCK in most organisms is the formation of PEP in the first committed step of gluconeogenesis. In leaves of Crassulacean acid metabolism (CAM) and C4 plants as well as in some diatoms, PEPCK functions as a decarboxylase involved in CO2-concentrating mechanisms. Since neither PEPCK function nor kinetic or regulation properties have been described in green microalgae, we decided to determine the physiological and biochemical role of PEPCK from Chlamydomonas reinhardtii (ChlrePEPCK). To this end, we analysed its sequence by comparison with homologous from other algae and plants: the four domains described for PEPCKs (PCK domain, kinase-1a domain, kinase 2 domain and ATP-binding motif) were highly conserved in ChlrePEPCK. Moreover, we built a homology model of ChlrePEPCK using the 3D structure of T. cruzi PEPCK (PDB code 1II2) as template finding that ChlrePEPCK model exhibits a fold similar to 1II2, with some differences in the ATP-binding motif. ChlrePEPCK was also cloned and purified to homogeneity and its biochemical properties were characterized. After studying its thermal and pH behaviour, we found that ChlrePEPCK carboxylates PEP with a hyperbolic response and maximum activity at pH 6 and 25°C. Our findings may contribute to a better understanding of PEPCK evolutionary process in the green lineage and to gain knowledge of its role in carbon metabolism.

EN-C03-144
DESIGN, SYNTHESIS, AND EVALUATION OF SUBSTRATE-ANALOGUE INHIBITORS OF *T. cruzi* RIBOSE 5-PHOSPHATE ISOMERASE TYPE B

Gonzalez SN, Mills JJ, Maugeri D, Olaya C, Laguera BL, Enders JR, Sherman J, Rodriguez A, Pierce JG, Cazzulo JJ, D'Antonio EL
Instituto de Investigaciones Biotecnológicas, Universidad Nacional San Martín-CONICET.
E-mail: gonzalezsolnat@gmail.com

Ribose 5-phosphate isomerase type B (RPI-B) is a key enzyme of the pentose phosphate pathway that catalyzes the isomerization of R5P and Ru5P. *Trypanosoma cruzi* RPI-B (*Tc*RPI-B) appears to be a suitable drug-target mainly due to: (i) its essentiality (as previously shown in other trypanosomatids), (ii) it does not present a homologue in mammalian genomes sequenced thus far, and (iii) it participates in the production of NADPH and nucleotide/nucleic acid synthesis that are critical for parasite cell survival. In this survey, we report competitive inhibition of *Tc*RPI-B by a substrate-analogue inhibitor,

Compound **B** (K_i = 5.5 ± 0.1 μM), by the Dixon method. On the other hand, after incubation with Compound **B**, which has an iodoacetamide group that is susceptible to nucleophilic attack, especially by a nearby cysteine sidechain (Cys-69) in the *Tc*RPI-B active site, in the absence of the substrate, trypsin digestion LC-MS/MS revealed the identification of Compound **B** covalently bound to Cys-69. This inhibitor also exhibited notable *in vitro* trypanocidal activity against *T. cruzi* infective life-stages co-cultured in NIH-3T3 murine host cells (IC_{50} = 17.40 ± 1.055 μM). The study of Compound **B** served as a proof-of-concept so that next generation inhibitors can potentially be developed with a focus on using a prodrug group in replacement of the iodoacetamide moiety, thus representing an attractive starting point for the future treatment of Chagas' disease.

EN-C04-207
INSIGHTS IN THE NADP+ BINDING MODE OF BACTERIAL FERREDOXIN-NADP+ REDUCTASES

Monchietti P, Ceccarelli EA, Catalano Dupuy DL
Instituto de Biología Molecular y Celular de Rosario (IBR), CONICET-UNR. E-mail: monchietti@ibr-conicet.gov.ar

Ferredoxin-$NADP^+$ reductases (FNR) constitute a family of proteins with a non-covalently bound FAD as a prosthetic group. They participate in redox metabolisms catalyzing the reversible electron transfer between NADP(H) and ferredoxin or flavodoxin. They are classified as plant- and mitochondrial-type FNR. Plant-type FNR are divided into plastidic and bacterial classes. The plastidic FNR show between 20- and 100-times higher exchange rates than bacterial enzymes. We have obtained experimental evidence that *Escherichia coli* FNR (EcFPR) contains its $NADP^+$ substrate tightly bound after isolation. The three-dimensional structure evidenced that $NADP^+$ interacts with three arginines (R144, R174 and R184) which could generate a site of very-high affinity and great structuring. These arginines are conserved in other bacterial FNR but not in highly efficient plastidic enzymes. Based on a structural alignment, we have cross-substituted EcFPR arginines with proline and tyrosine residues, which are present in analogous positions in the plastidic *Pisum sativum* FNR (PsFNR) (P199 and Y240) and replaced both amino acids with arginines in PsFNR. We analyzed all proteins by kinetic, structural, thermodynamic and stability studies. We discovered that, while EcFPR contains tightly bound $NADP^+$, its mutants lost the ability to bind the nucleotide, suggesting that mutations in Arg interfere with the $NADP^+$ binding site. Moreover, the mutants showed significantly increased K_M for $NADP^+$ and lower catalytic efficiencies than the wild-type enzyme. The activity of EcFPR was inhibited by $NADP^+$ but this behavior disappeared as arginines were removed. Unfolding studies showed that $NADP^+$ binding stabilized EcFPR structure. By using DMAP as analog of the nicotinamide portion of $NADP^+$ we observed an activation of EcFPR probably by releasing the tightly bound $NADP^+$ from the enzyme. On the other hand, we observed PsFNR did not bind $NADP^+$ and that the introduction of arginines in the PsFNR mutants was not enough to restore the bacterial $NADP^+$ binding site. This difference was further evidenced by the absence of any effect over kinetic parameters or structure stability by $NADP^+$ binding. Our studies indicate that the nucleotide binding characteristics between bacterial and plastidic FNR would be different and probably might be related with the differential catalytic efficiency observed. We propose that the high-affinity nucleotide binding is an essential catalytic and regulatory mechanism of bacterial FNR involved in redox homeostasis. This phenomenon might be used as a differential target for the inactivation of metabolic pathways in which the FNR participates in pathogenic bacteria.

NEUROSCIENCE

NS-C01-202
INTERNEURONAL EXCHANGE AND FUNCTIONAL INTEGRATION OF SYNAPTOBREVIN VIA EXTRACELLULAR VESICLES

Vilcaes AA[1,2,3], Chanaday NL [3], Kavalali ET [3,4]
[1]CIQUIBIC-CONICET. [2] Dpto de Qca Biológica R. Caputto-FCQ-UNC. [3] Department of Pharmacology. [4] Vanderbilt Brain Institute, Vanderbilt University- Nashville, TN-USA. E-mail: alejandro.vilcaes@unc.edu.ar

Recent studies have investigated the composition and functional effects of extracellular vesicles secreted by variety of cell types. However, the mechanisms underlying the impact of these vesicles on neurotransmission remain unclear. Here, we isolated extracellular vesicles secreted by hippocampal neurons and found that they contain synaptic vesicle-associated proteins, in particular the vesicular SNARE (soluble NSF-attachment protein receptor) synaptobrevin (also called VAMP). Using a combination of electrophysiology and live-fluorescence imaging, we demonstrate that this extracellular pool of synaptobrevins can rapidly integrate into the synaptic vesicle cycle of host neurons via a CD81- dependent process and selectively augment inhibitory neurotransmission as well as specifically rescue neurotransmission in synapses deficient in synaptobrevin. These findings uncover a novel means of interneuronal communication and functional coupling via exchange of vesicular SNAREs.