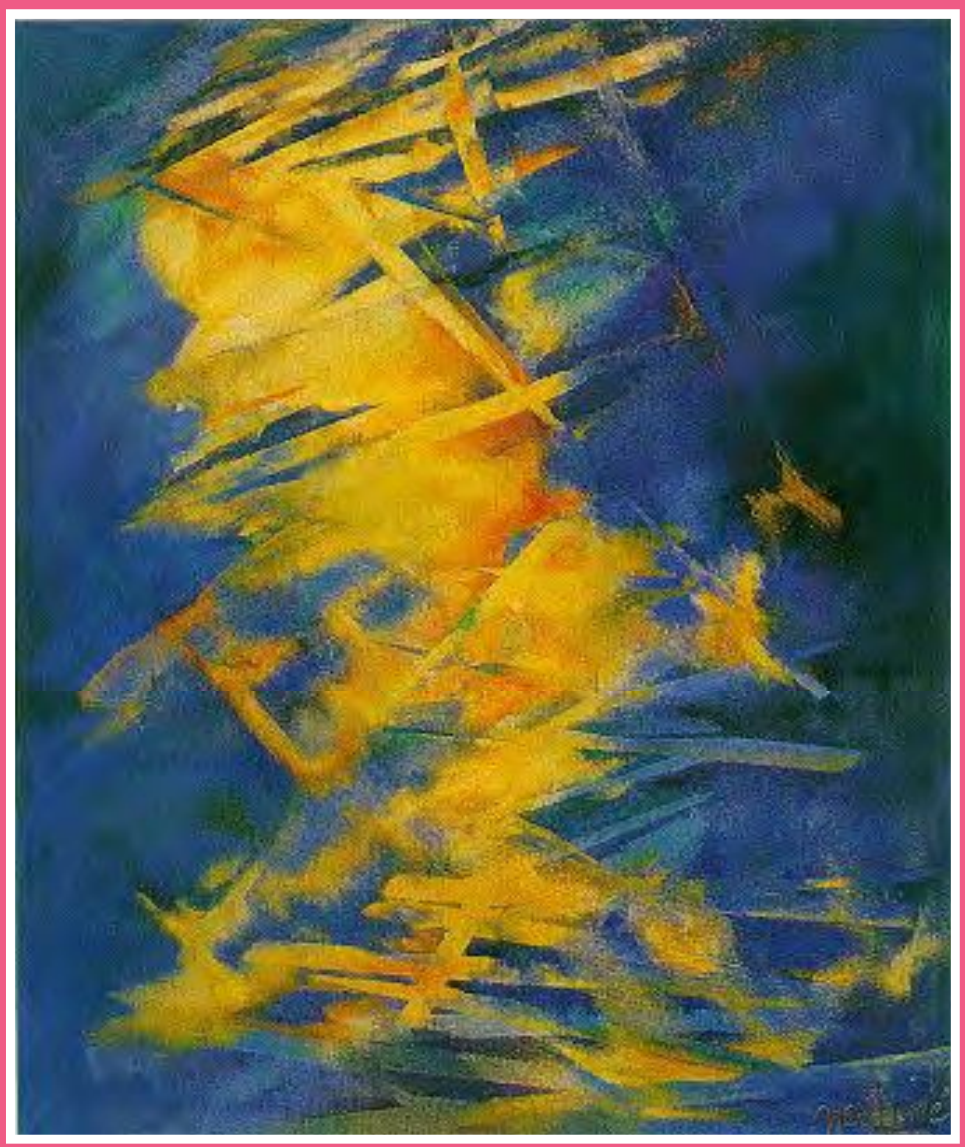
2017

medicina

BUENOS AIRES VOL. 77 Supl. I - 2017



MEDICINA

Volumen 77, Supl. I, págs. 1-620

phorylated intermediate which was found to be ADP sensitive. This suggests that EGCG stabilizes the *E*1P intermediate on the reaction cycle of hydrolysis of ATP by PMCA. In addition, while EGCG did not modify significantly the value of $K_{0.5}$ for Ca^{2+}, it increased the apparent affinity for Mg^{2+}.

In order to assess whether this inhibition may be of physiological relevance, we characterized this effect in the context of a living cell by monitoring in real time the changes in the cytosolic calcium levels. We tested the influence of EGCG on the activity of the PMCA transiently expressed in human embryonic kidney (HEK293) cells. We found that EGCG produced an increase of the cytoplasmic basal Ca^{2+} concentration and decreased the rate of removal of Ca^{2+} suggesting that PMCA activity was inhibited.

These results suggest that inhibition of the PMCA by EGCG can explain the observed effects on intracellular Ca^{2+} levels.

Keywords: Calcium, Epigallocatechin-3- gallate, inhibition, plasma membrane Ca^{2+}-ATPase

(1632) THE UNIQUE REDUCING STEP OF ORGANIC HYDROPEROXIDE RESISTANCE PROTEINS: THE CRUCIAL ROLE OF THE CATALYTIC ARGININE

Renato M. Domingos (1), Ari Zeida (2), William Agudelo (2), Raphael D. Teixeira (3), Thiago G. P. Alegria (1), Mario T. Murakami (4), Dario A. Estrin (2), Luis E. Netto (1)
(1) Department Genetics and Evolutive Biology IB, USP, São Paulo, Brazil. (2) Department of Inorganic Chemistry, Analytical and Physical Chemistry FCEN, UBA, Buenos Aires, Argentina. (3) Biozentrum UB, Basel, Switzerland. (4) National Center of Energy and Materials Research, Campinas, Brasil.

Organic Hydroperoxide Resistance proteins (Ohr) are Cys-based, lypoil-dependent peroxidases with extraordinary reactivity towards organic hydroperoxides (10^6–10^8 $M^{-1}s^{-1}$). These proteins are present in pathogenic microorganisms, exhibiting a unique barrel shaped fold and are absent in mammals. Such characteristics make Ohr promising drug targets. In the oxidative part of the catalytic mechanism, the peroxide is reduced via the oxidation of the reactive Cys at the active site yielding a sulfenic acid derivative; then a second Cys residue resolves, forming an intramolecular disulfide bridge. The reducing part of the cycle depends on a series of thiol/disulfide exchange reactions, which regenerates the reduced form of the enzyme. The catalytic triad of Ohr comprises a reactive Cys, an Arg and a Glu interacting in a closed state. In an open state, the loop containing the catalytic Arg moves away from the active site.

Here, we describe six new crystallographic structures (including the complex between Ohr and its biological reductant, dihydrolipoamide) that enable us to gain new insights on biochemical properties of Ohr. Furthermore, molecular dynamics simulations indicated that the most flexible regions of Ohr enzymes are located at the periphery of the active site, which might play a role in accommodating the different substrates. Moreover, when dihydrolipoamide was present at the active pocket, it provided additional stability to the close state of Ohr. Our results suggested that even upon disulfide bond formation, Ohr would have low probability of reaching the open state, represented in many Ohr structures available in PDB. In order to evaluate if the catalytic Arg is required to activate dihydrolipoamide, hybrid Quantum/Classical simulations are being performed, aiming to determine the free energy profiles regarding the reducing step, both in the open and close conformations. Taken these data together, an updated scheme for Ohr enzymatic mechanism is presented.

Keywords: Cys-based peroxidases, conformational changes, catalytic mechanisms, QM/MM.

(1905) ISOLATION OF A TRYPSIN-LIKE ENZYME FROM *Piaractus mesopotamicus* (PACÚ)

Laura Cristina Leiva (1,2), Gabriela Noemi Gómez (1,2), Bibiana Beatriz Nerli (2)
(1) LabInPro – FaCENA. (2) IQUIBA-NEA, UNNE, CONICET.

Piaractus mesopotamicus (pacú) is an omnivorous fish endemic of Paraguay-Paraná river basin, incorporated to the aquaculture production system in the northern region of Argentina. Fish viscera are a potential source of digestive enzymes, especially proteases. An interesting alternative to convert the fish processing wastes into useful products of higher value is the isolation of trypsin, one of the major digestive enzymes with potential industrial applications. This enzyme is currently marketed by its applications in food industry, as additive in laundry detergents, in cell culture technique and in the pharmacology area where is prescribed for several treatments (inflammatory edema associated with post-surgical wounds, traumatic injuries, rhinitis, sinusitis, etcetera). In this work we applied affinity chromatography on Benzamidine Sepharose column to isolate a tripsin-like protein from pacú processing waste. Pyloric caeca was disaggregated and homogenized in saline buffer (pH 7.8). After centrifugation, supernatant was applied to the column equilibrated in Tris buffer (pH 7.8). The adsorbed material was eluted with low pH buffers, 4.5 and 3.2. The eluents were monitored at 280 nm for proteins. Trypsin activity was measured by the change in absorbance at 410 nm using BApNA as substrate. Fractions collected at pH 3.2 which exhibited trypsin activity were pooled, dialyzed and lyophilized. SDS-PAGE showed a single band compatible with fish trypsins. Isolated protease was active in a wide range of temperatures (0–75°C) and the highest activity was found at 45°C. At 75°C, about 50% of maximum activity was retained, this value being 20% higher than that observed for commercial porcine trypsin. This remarkable property makes this enzyme an eligible ingredient in detergent manufacturing. Consequently, the single step method described above can be considered an useful tool to recover a trypsin-like enzyme from piloryc caeca of pacú for industrial purposes.

Keywords: pyloric caeca, serinoprotease, digestive enzyme, detergents

(676) BIOCHEMICAL CHARACTERIZATION OF AN EUCARIOTIC POLYKETIDE SYNTHASE

Martin Sabatini, Santiago Comba, Silvia Graciela Altabe, Ana Arabolaza, Hugo Gramajo
Instituto de Biologia Molecular y Celular de Rosario

Methyl-branched fatty acids (FA) are compounds which have numerous industrial applications. They show improved physicochemical properties in comparison to linear carbon chain FA, the most common FA found in natural sources. However, this kind of molecules is extremely rare in nature. Thus, with the aim to develop new compounds with optimized properties for industrial uses, in this work we explored a new strategy to generate structural diversity in FA.

Based on the mycoserosic acid synthase (MAS), a polyketide synthase (PKS) from *Mycobacterium tuberculosis* that synthesizes long chain methyl-branched fatty acids, we searched for a new PKS capable of using methylmalonyl-CoA (MMC) as extender unit. Using MAS as query for a BLASTp search, we identified a new family of un-characterized and highly conserved PKS in birds. The uropygial gland, in birds, is involved in the secretion of methyl-branched fatty acids, alcohols and esters that are used for cleaning and impermeabilization of the plumage. We hypothesized that these compounds would be produced by this new family of PKS. In particular, we started the characterization of a PKS enzyme, that we named ApMAS, from the duck *Anas platyrhynchos;* in this specie the uropygial secretion has a high proportion of methyl-branched- C6 FA.

PKS are enzymatic complexes that condense simple short chain acyl-CoA into larger molecules with diverse biological activities. In an initial biochemical analysis of ApMAS, we determined the ability of this enzyme to covalently bind to the substrates by using radiolabeled precursors, acetyl-CoA/propionyl-CoA as starter units and malonyl-CoA/MMC as extender units. Using this strategy we could also evidence the substrate transfer from an acyl-CoA to the ApMAS acyl carrier protein domain (ACP). We then measured the kinetics of this transfer reaction catalized by the ApMAS acyltransferase domain. Finally, we could demonstrate the *in vitro* condensing ability of the ApMAS ketosynthase domain.

Palabras clave: methyl-branched fatty acids, polyketide synthase

(869) BIOCHEMICAL CHARACTERIZATION OF THIOREDOXIN DEPENDENT CELLULAR PATHWAYS IN *Trypanosoma cruzi*