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ABSTRACTS

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Proline Transporter ProP of *Escherichia coli*: Osmosensor and Osmoregulator

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Osmotic pressure fluctuations perturb diverse aspects of cell physiology, including cell shape, volume and turgor pressure, cytoskeleton and wall organization, cytoplasmic membrane shape and tension, macromolecular structures and interactions, intracellular solute composition, solute and solvent activities. Cells respond to variations in osmotic pressure (Π) and temperature (T) by modulating their composition. As increasing external osmolality (Π/RT) elicits dehydration, organic osmolyte accumulation stabilizes cytoplasmic macromolecules and elicits rehydration. Decreasing osmolality elicits osmolyte release. Well-characterized transporters, enzymes and mechanosensitive channels modulate the osmolyte composition of Gram negative bacterium *Escherichia coli*. Exploiting that system, we aim to understand how osmotic pressure is sensed, how resulting signals are transduced, and how cells respond to modulate their structure, growth and division. ProP of *E. coli* was the first protein recognized as an osmosensor: it activates as osmotic pressure increases, both in vivo and after purification and reconstitution in proteoliposomes. ProP is a member of the Major Facilitator Superfamily and a H^+-solute symporter. This seminar will address structure-function relationships fundamental to osmosensing by ProP. It will also show that an α-helical coiled-coil forming ProP domain, membrane cardiolipin content, and localization of ProP with cardiolipin at *E. coli* cell poles all influence the osmolality range to which ProP responds. For additional information, see a perspective on *Osmosensing by Bacteria* (Science's STKE, 17 October 2006, Vol. 2006, Issue 357, p. pe43).

Inborn Errors of Proline and Ornithine Metabolism

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Our understanding of proline and hydroxyproline metabolism, biological functions and disease associations has increased dramatically since the first recognition of direct involvement of proline and hydroxyproline in human disease 45 years ago (Schafer, Scriver and Efron, NEJM 267: 51, 1962). At least 8 enzymes, 3 transporters and 11 structural genes are directly involved in the interconversions of proline, hydroxyproline and their immediate metabolites. The functional characteristics, tissue distribution, regulation and subcellular location of these proteins have, for the most part, been determined and structural information is available for some.

In addition to its role as a protein amino acid, proline serves as an osmolyte in certain cells and in the CNS has features of a neuroinhibitory neurotransmitter as well as being a metabolic precursor for glutamate in glutamatergic synapses. Recent studies have shown that *PRODH* (22q11.2), which encodes proline oxidase (POX) the first enzyme in the proline catabolic pathway, is highly polymorphic with multiple variants with a spectrum of consequences on enzyme activity. *OHPRODH* (19q3.12), encodes hydroxyproline oxidase and appears to have far fewer polymorphic variants. *PRODH* is one of a handful of genes whose expression is induced by p53. Subsequent work has shown that the increased amounts of POX together with availability of proline results in increased reactive oxygen production that can lead to apoptosis and alterations of cell cycle. These perturbations may play a key role in oncogenesis in certain cells and tissues.

Seven monogenic inborn errors of metabolism and/or transport involving proline, hydroxyproline and their immediate metabolites have been described and mouse models are available for some of these. Moreover, impaired proline metabolism has been implicated as a susceptibility factor for schizophrenia, a complex neuropsychiatric disorder with a frequency of $\sim 1\%$ around the world.

I will provide an overview of our current understanding of these inborn errors, what they tell us about proline and hydroxyproline metabolism and its relationship to common complex phenotypes.

MYC: Oncogenic Alterations of Intermediary Metabolism

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The protooncogene *MYC* encodes a transcription factor, Myc, which regulates cell size, cell proliferation, cell cycle progression, apoptosis and metabolism. We provide a snapshot of direct Myc binding sites through an unbiased, genome-wide approach that couples chromatin immunoprecipitation with pair-end ditag sequencing analysis (ChIP-PET) in a B cell model of Burkitt lymphoma. Myc potentially occupies more than 4000 loci with a major fraction near proximal promoter regions. Among the 4,000 putative sites, about 2,000 are considered high-quality binding sites. By correlating with gene expression profiling, we identified 668 direct Myc-regulated target genes that span a variety of cellular functions. The use of high quality Myc binding sites permitted the bioinformatic identification of transcription factor consensus binding sites, thereby providing a glimpse of cis-regulatory modules regulated by Myc and other transcription factors such as E2F1. We identified putative Myc binding sites that are associated with microRNAs, which are both down-regulated and upregulated by Myc. Functional studies indicate that miRNAs are important mediators of Myc function. Furthermore, our studies pointed to a role for Myc in regulating intermediary metabolism, such as the induction of ornithine aminotransferase, as well as confirming the previous links between Myc and glucose metabolism. The role of Myc in hypoxia is intriguing since the hypoxia inducible factor 1 (HIF-1) is required for Myc-mediated tumorigenesis. Furthermore, we found an expected anti-tumorigenic effect of antioxidants, vitamin C and *N*-acetylcysteine, through their ability to reactivate prolyl hydroxylase to trigger the degradation of HIF-1α. These studies underscore the role of Myc as a master regulator of cellular function and the link between cell cycle progression and cellular metabolism.

The Role of Glutamate in the Pathophysiology of Schizophrenia

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After 50 years of antipsychotic drug development focused on the dopamine D2 receptor, schizophrenia remains a chronic, disabling disorder for most affected individuals. Studies over the last decade demonstrate that administration of low doses of antagonists for the NMDA subtype of glutamate receptor can cause in normal subjects the negative symptoms, cognitive impairments and physiologic disturbances observed in schizophrenia. Furthermore, a number of recently identified risk genes for schizophrenia affect NMDA receptor function or glutamatergic neurotransmission. A sub-group of parvalbumen-expressing GABAergic neurons in the intermediate layers of the cortex are most sensitive to NMDA receptor antagonists and are similarly affected in schizophrenia. Placebo-controlled trials with agents that directly or indirectly activate the glycine modulatory site on the NMDA receptor have shown reduction in negative symptoms, improvement in cognition and in some cases reduction in

positive symptoms in schizophrenic patients receiving concurrent antipsychotic medications. Thus, hypofunction of the NMDA receptor, specifically on critical GABAergic inter-neurons, contributes to the pathophysiology of schizophrenia.

Proline and PRODH in Schizophrenia

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Schizophrenia (Sz) is a common (1% incidence), debilitating neuropsychiatric disorder with a peak age of onset at the end of the second decade. Despite profound abnormalities of brain function, pathological examination shows no neurodegeneration or other diagnostic abnormalities in the central nervous system. Current models of the pathophysiology of Sz suggest that it is a neurodevelopmental disorder with abnormalities of the glutamate system of neurotransmission.

Family studies with a sibling relative risk of 10% and an MZ/DZ twin concordance rate of 50/10% strongly support a major role for genetic factors in Sz. Despite this evidence and two decades of intense investigation, relatively little is known about the genetic factors contributing risk for Sz. There is strong evidence for 2 or 3 genes (*DISC1, NRG1*) and suggestive evidence for about 10 more. One of the latter is *PRODH*, which encodes proline oxidase and is located at 22q11.2. This region of the genome is included in the common (1/4000 live births) 3 Mb deletion that includes more than 70 annotated genes and is the cause the DiGeorge/velocardiofacial or 22q- syndrome. More than a decade ago, Pulver and her colleagues showed that individuals with the 22q- syndrome had an $\sim$25-fold increased risk of developing Sz.

The 22q- observation suggested that a gene or genes in this region contributed risk for Sz. Additional observations implicating *PRODH* include: recognition of possible roles for proline as a neurotransmitter and precursor of glutamate in the CNS; identification of a large number of common (>0.01) *PRODH* variants with variable functional consequences, some of which have been associated with Sz in certain studies; the discovery of type 1 hyperprolinemia (HP1) patients with Sz; and, the observation that a mouse model with HP1 has neurobehavioral phenotypes consistent with those of humans with Sz.

Despite this evidence supporting a role for *PRODH* variants contributing risk for Sz, other studies have not found an association. These conflicting data emphasize the need for additional studies of the function of proline and *PRODH* in the CNS and new technologies to examine the in vivo consequences of *PRODH* variants on CNS function. Elucidation of any role for *PRODH* in Sz may also have to take the individual context into consideration; i.e. the risk conferred by *PRODH* variants may depend on the individual's genotype at other loci and on their environmental history.

POX Imaging Genetics: Proline Oxidase's Impact on Brain Structure and Function

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PRODH, encoding proline oxidase (POX), is a gene on 22q11.2 with previous associations with a missense snp with schizophrenia in several populations. Also, 22q11.2 deletion patients with disruption of proline metabolism have been reported to have increased risk for psychosis and hyperprolinemia has been associated with psychosis and neurological disorders. Fortunately, functional polymorphisms that determine the relative activities of the enzyme have been recently described that enable a genetic approach to probe the possible role of POX in brain schizophrenia. We tested several of these common functional polymorphisms in our clinical dataset to test for association with schizophrenia. Additionally, to separate the individual effects of the polymorphisms, we genotyped a large population of psychiatric and neurologically screened subjects and formed functional haplotypes to examine the genotypic effects on brain structural and working memory with FMRI neuroimaging. We found that the polymorphism that increases POX activity also increased risk for schizophrenia and polymorphisms that decrease POX activity decreased risk. This differential clinical association was mirrored by dissociable effects on neural intermediate phenotypes: the risk haplotype decreased striatal grey matter volume, and impacted on the bottom-up processing stream in working memory through increased striatal frontal functional connectivity, whereas the protective haplotype increased frontal grey matter with a shift to increased posterior to anterior attentional processing in working memory and decreased striatal frontal functional connectivity. This association suggests a role for genetic variation in proline metabolism on neostriatal-frontal circuits mediating risk for schizophrenia.

Functional Food Design via Proline-linked Redox Pathways to Counter Diet-Linked Chronic Disease Challenges

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Diet-linked chronic diseases associated with metabolic syndrome such as Type 2 diabetes and cardiovascular diseases are changing the face of global disease burden. Since these are oxidation-linked chronic diseases and preventable, solutions require "outside the box" innovations based on understanding of genomic and metabolic questions along with integrating concepts of redox biology. How designed diet modulates human redox-protective and inducible metabolic pathways is key to chronic disease prevention and management. The same rationale of redox-protective inducible pathways in humans has been targeted for food and ingredient design from food plants, animal foods and microorganisms, which also incorporates traditional whole diet and food diversity for practical solutions. The understanding of synthesis of phenolic redox protective ingredients is integrated with proline metabolism of diverse food systems. The rationale of proline-linked redox responses is also important for host responses to designed phenolic metabolites. This proline-linked redox link will be highlighted using metabolic syndrome and breast cancer models. This approach has implications for meeting the challenges of human health and wellness while at the same time adding high value to sustainable food and agricultural systems.

Structures of the DNA-binding Domain of the Multifunctional *Escherichia coli* PutA Flavoprotein

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Proline is metabolized in *Escherchia coli* by the multifunctional flavoprotein Proline Utilization (PutA) protein. In the presence of

proline, PutA functions as a membrane-associated enzyme catalyzing the two-step oxidation of proline to glutamate. In the absence of proline, PutA accumulates in the cytoplasm and functions as transcriptional repressor of its own gene and the gene for the PutP Na^+/proline transporter. The *E. coli* PutA protein is a challenging structural biology target due to its large size (1,320 amino acid residues), multidomain architecture (three domains), inherent flexibility, and peripheral association with membranes. Extensive crystallization trials failed to produce a usable crystal form for structure determination. Therefore, molecular dissection, a molecular biology based approach, was employed. Molecular dissection led to high quality crystals of the free and complexed DNA-binding domain (PutA52, amino acid residues 1–52). The 2.1 Å resolution structure of PutA52 mutant Lys9Met was determined using Se-Met MAD phasing, and the structure of native PutA52 was solved at 1.9 Å resolution using molecular replacement. Residues 3–46 form a ribbon–helix–helix (RHH) substructure, establishing PutA as the largest protein to contain an RHH domain. PutA52 was co-crystallized with a 21-base pair DNA fragment corresponding to one of the five PutA binding sites in the put control region. A 2.25 Å resolution data set was used to solve the PutA52/DNA structure with molecular replacement using PutA52 dimer as the search model. The current model consists of one PutA dimer (87 residues), 37 of the expected 42 nucleotides, and 11 water molecules. DNA-binding characteristics of the native PutA protein and PutA52 are compared by isothermal titration calorimetry (ITC).

Interactive Role of POX and P66^{Shc} in Oxidative Stress-induced Cell Death in Cancer Cells

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Proline oxidase (POX), a mitochondrial inner membrane protein, catalyzes the rate-limiting oxidation of proline to pyrroline-5-carboxylate using cytochrome c (cyt c) and FAD as electron and hydrogen acceptors. Previously, we showed that POX is one of the p53 downstream effectors that induces ROS- and mitochondria-mediated apoptosis in cancer cells. We also showed that POX initiates both intrinsic and extrinsic apoptotic pathways, possibly through NFAT and MEK/ERK signaling. P66shc, a 66-kDa isoform of Src homologue and collagen homologue (shc) adaptor family, has been shown to be involved in ROS-mediated apoptosis in response to oxidative stress. Although p66^{shc} localizes predominantly in the cytosol, in response to oxidative stress signals, a fraction of p66^{shc} is phosphorylated leading to a conformational change that targets phosphorylated p66^{shc} to the mitochondria inter-membrane space. Phosphorylated p66^{shc} interacts with mtHsp70 and other proteins to mediate apoptosis. Importantly, p66^{shc} also is a downstream target of p53. It has been shown that p53-p66^{shc} pathway functions as a sensor of intracellular oxidative status and a regulator of intracellular oxidants and therefore apoptosis. Moreover, p66^{shc} has been shown to interact with reduced cyt c to produce H_2O_2, which can promote opening of the mitochondrial permeability transition pore that ensures release of cyt c to induce apoptosis. Finally, a recent study showed that p66^{shc}-induced apoptosis can also be regulated by mitochondrial energetic conditions and abnormal expression of p66^{shc} has been described in breast cancer cells. We hypothesize that POX and p66^{shc} have interactive and synergistic effect on the production of ROS in apoptosis in cancer cells. We have generated cancer cells overexpressing POX and p66^{shc} and will use magnetic nanoparticles, affinity chromatography, yeast two hybrid system, functional assays and proteomic strategy to dissect possible interaction and synergistic effect between p66^{shc} and POX in generation of ROS and induction of cell death.

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Deletion of v7-3 (SLC6A15) Transporter Improves Motor Performance in Aging Female Mice

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V7-3 (SLC6A15) is the prototype for a gene subfamily whose members have sequence homologies to classical Na^+- and Cl^--dependent neurotransmitter transporters but display unusual features including characteristic large fourth extracellular loops. Interest in v7-3 has been increased by elucidation of its expression in neurons located in cerebral cortex, hippocampus, cerebellum, midbrain, and olfactory bulb. To help clarify the role of v7-3 in brain functions, we have created and characterized v7-3 knockout mice. These mice lack functional v7-3 protein, but are viable and fertile. While our studies were in progress, v7-3 expression was reported to confer transport of proline and branched-chain amino acids in in vitro expression systems. Assessment of amino acid uptake into cortical synaptosomes of v7-3 knockouts identifies 15 and 40% reductions in sodium-dependent proline and leucine transport, respectively, compared to wild type controls. Despite these biochemical changes, v7-3 knockout mice demonstrate lack of reproducible alterations in health, reproduction, memory, anxiety or olfactory tests. The only exception was that mature homozygous v7-3 KO females consistently outperformed their wild type siblings on the accelerating rotarod task. The current results place v7-3 in the context of other brain transporters that accumulate proline and branched-chain amino acids, and point to the specific role of the v7-3 transporter in cerebellar function.

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Rhodiola crenulata-induced Cell Death in Breast Cancer Cell Line Involves Dysfunctional Mitochondrial Oxidative Phosphorylation Associated with Proline-linked Pentose Phosphate Pathway

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Drugs and Natural compounds have potential for chemoprevention of breast cancer. Evidence indicates that drugs such as tamoxifen, anastrozole and several plant-derived compounds such as ellagic acid, curcumin and caffeic acid inhibit some stages of breast cancer growth. For more effective inhibition of cancer cell growth critical control points that control cellular metabolism towards overall cellular energy breakdown is essential. In this study, phenolic phytochemicals from *Rhodiola crenulata* extract triggered apoptosis in the V14a breast cancer cell line by excessively increasing reactive oxygen species and forcing higher activity of antioxidant enzymes such as superoxide dismutase, catalase and myeloperoxidase. Our hypothesis is that certain breast cancer cells have dysfunctional antioxidant pathways linking critical mitochondrial-based proline oxidation with cytosolic pentose phosphate pathways (PPP). This

proline-linked pathway activates protective antioxidant pathways in response to phytochemicals in normal cells. In order to survive, breast cancer cells likely avoid this dysfunctional proline-linked antioxidant pathway. Evidence from this study indicates that by forcing *Rhodiola*-induced switch back to dysfunctional proline-linked pathway, breast cancer cell death can be induced via inhibition of dehydrogenases such as Krebs cycle-linked succinate dehydrogenase and also alternative energy-linked proline dehydrogenase. Further the proline-linked antioxidant response can be disrupted. In this breast cancer cell line, the *Rhodiola* treatment activated the dysfunctional mitochondrial oxidative phosphorylation linked to proline synthesis but was starved of energy due to lack of proline utilization by the inhibited proline dehydrogenase. These likely results in uncoupling of antioxidant response forcing dysfunctional cell to be overwhelmed by reactive oxygen species (ROS).

First Crystal Structure of a Full-Length PutA: Evidence for Substrate Channeling

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In some bacteria, proline dehydrogenase (PRODH) and P5C dehydrogenase (P5CDH) are combined into a single protein known as Proline utilization A (PutA). Fusion of enzymes that catalyze sequential steps in a pathway offers several metabolic advantages, including substrate channeling and sequestration of reactive and/or toxic intermediates. Although crystal structures of some of the functional domains of PutAs have been solved, the structure of full-length PutA has remained elusive. This poster will describe the first crystal structure of a full-length PutA, which has been determined at a resolution of 2.1 Å. The PRODH and P5CDH active sites are separated by over 40 Å. There is a large, solvent inaccessible cavity that connects the two active sites and we propose that this cavity serves as a substrate channel. This structure, together with biochemical studies, supports the hypothesis of substrate channeling in PutA.

Building the Wire in *E. coli* PutA

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The research goals and objectives for my project are to mainly understand the overall structural wiring of the *Escherichia coli* PutA (EcPutA) protein upon reduction. PutA (proline utilization A) oxidizes free proline. When EcPutA is reduced, studies show that it binds to the membrane of the cell; however, the oxidized form of EcPutA will bind to DNA. Recent studies show that with a reduction in the EcPutA FAD cofactor, an increase in EcPutA-lipid binding affinity is observed. Therefore, it can be concluded that FAD reduction is critical for EcPutA membrane binding. A critical residue, Arginine 431 (R431), in the EcPutA protein is important for forming a stabilizing FAD N(5)-R431 bond. When this bond is disrupted, a conformational change in the protein occurs resulting in EcPutA-lipid membrane binding. The entire EcPutA protein sequence has not been solved so it is unclear what other residues are important in the active site of this FAD cofactor. Thus, it is important to find out other important residues in EcPutA that are critical for this conformational change. Here we identified three residues (D370, S327 and Q511) that hydrogen bond to the R431 residue. Therefore, quick change site directed mutagenesis was performed to find out if any of these three mutants affect the overall affinity of EcPutA protein membrane binding. To investigate this, cell based reporter gene assays were performed and indicated decreased reduction of proline in a dose dependent manner. Thus, the EcPutA protein remained bound to the DNA. Thus, a structural wiring can be resolved critical for EcPutA-membrane interactions. We also purified one of the three mutants, S327A, to start characterizing the protein. Kinetic assays, ProDH, Kcat and Km were found building a hypothesis for how this hydrogen bond triad contributes to the FAD redox switch. Outcomes from this project can lead to further understanding and drug development for proline bioenergetics in cancer, trypanosomal diseases and schizophrenia.

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Exploring the Membrane Binding Properties of PutA from *Escherichia coli*

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PutA is a multifunctional flavoenzyme from *Escherichia coli* that catalyzes the oxidation of proline to glutamate through a two-step catalytic process. PutA is composed of a single 1,320 amino acid polypeptide which contains two separate catalytic sites involved in proline oxidation as well as transcriptional repression properties mediated by a N-terminal ribbon-helix-helix (RHH) domain. In addition, PutA is known to have peripheral membrane binding properties, allowing for the transfer of reducing equivalents to the respiratory chain, carried out by an, as of yet, unknown mechanism. Current efforts have been directed toward characterizing the membrane binding domain(s) of PutA, which defines the objective of this study. Peripheral membrane binding proteins typically bind phospholipids through a combination of electrostatic and hydrophobic forces. Since native phospholipid headgroups commonly have a negative charge, electropositive residues are mostly responsible for the initial protein to membrane attraction which may be further stabilized by a partial penetration of one leaflet of the bilayer by hydrophobic or amphiphilic amino acid residues. The side chains of arginine and lysine residues contain an aliphatic carbon chain ending with a positively charged nitrogen containing functional group. These amphiphilic residues are thought to be able to "snorkel" the membrane medium creating a shallow anchor for the protein. Arginine 425 in *E. coli* PutA is an example of a potential membrane "snorkeling" target for mutagenesis studies. Substitution of arginine 425 to alanine (R425A) has resulted in an average decrease in membrane binding according to functional membrane binding assays. Additional experiments with R425A and other mutant PutA proteins (R423A + R425A and PutA630R425A) with techniques such as surface plasmon resonance and hydrophobic photolabeling will be used to help characterize the potential membrane binding domains of PutA.

A Microarray Analysis for Differential Gene Expression for Two Cancer Cell Lines, DU145 Non-drug resistant and RC.01 Drug-resistant Using R & bioconductor

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This research performs specific procedures for conducting quality assessment/quality control of Affymetrix GeneChip® cancer genome data. We have conducted biostatistical analyses to determine differential gene expression using the open-source R language in

conjunction with the open-source Bioconductor packages. We describe and demonstrate the use of exploratory plots including images of raw probe-level model, box plots, density plots, and M versus A plots. RNA degradation and recommended procedures from Affymetrix for quality control are discussed. We have used the Robust Multichip Averaging (RMA) procedure for background correction, normalization and summarization of the AffyBatch probe level data to obtain expression level data and to discover oncogenes that are differentially expressed.

NONO-NSAIDS Possessing a PROLI/NO Moiety: The New Anti-Inflammatory, Analgesic, and Potentially Chemopreventive Prodrugs

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A novel group of PROLI/NO-based non-steroidal anti-inflammatory prodrugs (NONO-NSAIDs) were synthesized and screened for anti-inflammatory and analgesic activities. The resulting nitric oxide (*NO)-releasing prodrugs CVM-04 and CVM-07 significantly decreased carrageenan-induced rat paw edema showing enhanced in vivo anti-inflammatory activities (ID50's = 314 and 18 μmol/kg, respectively) relative to those of the parent NSAIDs aspirin (ID50 = 714 μmol/kg), and indomethacin = 11.8 μmol/kg). The rate of porcine liver esterase-mediated *NO release from prodrugs CVM-04 and CVM-07 was substantially higher compared to that observed without enzymatic catalysis. These incubation studies suggest that both *NO and the parent NSAID would be released upon in vivo activation (hydrolysis) by esterases. Data acquired in an in vivo ulcer index (UI) assay showed that NONO-aspirin (UI = 0.75), and NONO-indomethacin (UI = 0) were significantly less ulcerogenic compared to the parent drugs aspirin (UI = 51) or indomethacin (UI = 64) at equimolar doses. Since NONO-NSAIDs are practically devoid of gastric toxicity, their use may constitute a promising alternative for patients taking classical NSAIDs but diagnosed with gastropathy, or for patients at high risk for coronary artery disease taking selective COX-2 inhibitors. NONO-aspirins may also provide a promising alternative to the use of aspirin as an anti-thrombotic agent in the long-term prophylactic prevention of stroke and myocardial infarction, or as a safer chemopreventive agent for colorectal cancer.

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L-Lactic Acid Induced Cell Death in *Listeria monocytogenes* Involves Proline-linked Dysfunctional Glucose-6-Phosphate Dehydrogenase and Catalase-associated Antioxidant Response

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Listeria monocytogenes is a food safety challenge in fresh and frozen meat and poultry. Natural antimicrobials such as phenolic phytochemicals and natural organic acids and their salts are currently used as antimicrobial strategies. To develop improved strategies for *L. monocytogenes* control, a better understanding of the cellular energy and antioxidant pathways are essential. In this research the efficiency of various concentrations of L-lactic acid were evaluated for *L. monocytogenes* control. A dose dependent cell-death was observed at all tested concentrations. Furthermore, results indicate that L-lactic acid may promote cell death by inhibition of catalase (CAT) and glucose-6-phosphate dehydrogenase (G6PDH). This inhibition of G6PDH by L-lactic acid likely prevents NADPH generation and affects ATP levels. This likely shifts energy dependency to both tricarboxylic acid (TCA) pathway and to proline oxidation as indicated by increased succinate dehydrogenase (SDH) and proline dehydrogenase (PDH) activities. The resulting enhanced proline content and likely reactive oxygen species (ROS) pressure from both proline accumulation and reductants from TCA cycle could be lethal coupled with the inability of catalase to counter ROS. This indicates that L-lactic acid can disrupt the proline-linked antioxidant counter measures, while concurrently over-expressing energy producing pathways that may not be supported by purine synthesis for ATP.

Crystal Structure of Human Pyrroline-5-carboxylate Reductase

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Pyrroline-5-carboxylate reductase (P5CR) is a universal housekeeping enzyme that catalyzes the reduction of 1-Pyrroline-5-carboxylate (P5C) to proline using NAD(P)H as the cofactor. The enzymatic cycle between P5C and proline is very important for the regulation of amino acid metabolism, intracellular redox potential, and apoptosis. Here we present the 2.8 Å resolution structure of the P5CR apo-enzyme, its 3.1 Å resolution ternary complex with NAD(P)H and substrate-analog. The refined structures demonstrate a decameric architecture with five homodimer subunits and ten catalytic sites arranged around a peripheral circular groove. Mutagenesis and kinetic studies reveal the pivotal roles of the dinucleotide-binding Rossmann motif and residue Glu221 in the human enzyme. Human P5CR is thermostable and the crystals were grown at 37°C. The enzyme is also implicated in oxidation of the anti-tumor drug thioproline.