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ABSTRACTS

Department of Biology

Apoptotic Inhibition of C3H-10T1/2 Mouse Embryonic Fibroblasts Due to the Overexpression of the Baculovirus Gene, p35.

Kimberly D. Burnette-Deaton.
Faculty Mentor: Dr. Steve Runge

Apoptosis is a naturally occurring cell death that rids the body of unneeded or unhealthy cells. Although the process is not completely understood, it has been shown that the process is genetically controlled. Apoptosis can be stimulated by a variety of mechanisms, such as chemotheraputic drugs, calcium influx, and hormonal withdrawal. The apoptotic process can also be inhibited by many diverse factors, as well. The most significant of these factors appears to be genes. Our gene of interest is p35. This gene is found in the insect baculovirus, Autographa californica multiply-embedded nuclear polyhedrosis virus (AcMNPV) and produces a protein that is a known inhibitor of apoptotsis. The mechanism by which p35 inhibits apoptosis is not yet clear, but recent findings suggest that it may function as an inhibitor of the ICE family proteases. ICE is the mammalian cysteine protease interleukin-1-beta-converting enzyme. Research has shown that ICE induces apoptosis when it is overexpressed. However, in ICE-deficient mice, apoptosis occurs indicating that other mechanisms of inducing cell death are available to the cell. The mammalian cell chosen for this experiment is the mouse embryonic fibroblast cell strain C3H-10T1/2. The p35 gene has been stably transfected into this cell line and seven cell clones were isolated. Each clone is resistant to the induction of apoptosis induced by serum withdrawal, but their level of resistance is variable. The purpose of this project is to determine if the variable resistance is due to the variable levels of expression of the p35 gene.

Effect of alanine on apical membrane endocytosis inAplysia intestinal cells

Jamie Daniel
Faculty Mentor: Dr. Mike Moran

After a meal, sugars and amino acids enter nutrient absorbing enterocytes by Na+-coupled mechanisms located in the luminal (apical) membrane. Rapid entry of osmotically active Na+ ions, sugar molecules, and amino acid molecules increases intracellular osmolarity. The problem of intracellular solute loading is compounded because the increase in intracellular Na+ concentration stimulates activity of the basolateral membrane Na+, K+-exchange pump which, in turn, stimulates K+ entry across this barrier. The increase in intracellular osmolarity causes osmotic cell swelling, threatening the enterocytes with extinction. To counteract the osmotic effect, enterocytes increase plasma membrane K+ conductance, which stimulates efflux of K+ ions and returns cell volume to pre-nutrient levels. This response ensures that nutrient molecules are absorbed at normal rates. We have been using the marine snail (Aplysia californica) intestine as a

model system to investigate the mechanism that increases the number of active plasma membrane K+ channels which comprise membrane K+ conductance. We have shown that luminal alanine increases apical membrane K+ conductance in Aplysia enterocytes (Gabbard and Moran, 1995. Am. J. Physiol.268 (Regulatory Int. Comp. Physiol. 37):R1050-9). However, in no nutrient or Na+-absorbing epithelium is the nature of the increase in the number of active K+ channels known. In the past, ion channels were thought to be activated in situ in the plasma membrane. However, recently a second mechanism has become recognized: exocytic insertion of channels into plasma membranes from intracellular vesicles located just beneath the barrier. Increases in exocytic insertion, however, can occur by two different but related pathways: 1) increase in exocytic insertion, as stated above, or 2) decrease in endocytic retrieval from the plasma membrane. A decrease in endocytic retrieval will increase the number of apical membrane K+ channels because their residence time in the membrane is extended, and constitutive exocytosis continues to deliver K+ channels to this barrier. In this study, we will assess the effect of alanine absorption on endocytosis in Aplysia enterocytes. We predict that alanine will decrease endocytosis of K+ channels and that this contributes to the increase in the number of functional K+ channels in the enterocyte's apical membrane. Our first experimental objective was to determine the endogenous and surface-bound activity of the enterocyte. Our results show that the average native peroxidase activity is 0.0139 abs/min (SD 0.0012). The surface-bound (nonspecific) HRP activity averages 0.031 abs/min (SD 0.0072). We will now be able to use these data to reveal endocytic uptake of HRP in future experiments.

Does galactose increase apical membrane K+ conductance in seahare enterocytes?

Kelli Wilson
Faculty Mentor: Dr. Mike Moran.

Survival of all animal cells requires maintenance of intracellular solute concentrations and cell volumes within very narrow ranges. This aspect of cellular homeostasis is a problem for nutrient-absorbing intestinal epithelia, which absorb nutrients by Na+-coupled mechanisms located in the enterocyte's apical membrane: After a meal the nutrients stimulate Na+-coupled nutrient entry across the apical (luminal) membrane and "flood" the cell with these osmotically active solutes. This rapidly increases intracellular ion concentrations and cell volume by osmosis. The problem of intracellular solute loading is compounded because the increase in intracellular Na+ concentration stimulates basolateral membrane Na+,K+-exchange pump activity which, in turn, increases K+ entry across this barrier. Without compensatory mechanisms to regulate cell volume, nutrient absorption by intestinal enterocytes would be accompanied by large, sustained increases in enterocyte volume that would interfere with the process of nutrient transport. We developed the seahare (Aplysia californica) intestine as a simple model system to test the hypothesis that nutrient absorption increases apical membrane K+ conductance (Moran and Garretson, 1988). Conventional glass microelectrode techniques were used to study the effect of alanine on cellular electrophysiology of isolated Aplysia intestine. Superfusion of the luminal surface with 10 mM alanine causes an abrupt depolarization (cell interior becomes electrically more positive) of apical membrane potential (Va) because alanine stimulates Na+ entry across the

apical membrane. This is followed by a gradual (~ 5 min) 10 mV repolarization of Va due to increased apical membrane K+ conductance which stimulates K+ efflux and reduces the number of osmotically active osmolytes in the cytosol (Gabbard and Moran, 1995). The increase in K+ conductance ensures that nutrient molecules are absorbed by the epithelium at normal rates. However, the generality of this hypothesis may not apply to all classes of nutrient molecules because 10 mM galactose (a monosaccharide) elicits either a small repolarization (2-3 mVs) of Va or no repolarization at all following Na+/galactose-induced depolarization. Further, K+ jump experiments show no increase in apical membrane K+ conductance as has been observed with alanine. These findings suggest that galactose is rapidly metabolized and does not accumulate in the cytosol as an osmotically active solute particle. If so, this would imply that rapid intracellular accumulation of nutrient molecules represents the "signal" that increases apical membrane K+ conductance.

Department of Chemistry

Determination of Reaction Energetics in a Drift Cell Reactor via Heavy-Ion Mobility Studies

D.F. Barnas and E.M.Spicer
Faculty Mentor: Dr. W.S. Taylor

The kinetic and internal energy distributions of bare metal ions can strongly influence the outcomes of their interactions with neutral molecules. This study focuses on the measurement of ion mobilities, which are used as a means of examining the energetics of Ta^+, Pt^+, and Au^+ in a helium bath gas. The energetics of the ions are affected by both the ionization process as well as the conditions present in the bath gas. Our apparatus consists of a glow discharge ion source, a drift cell ion reactor, and a quadrupole mass analyzer. Instrumental parameters influencing both the kinetic energy and the internal energy of the ions have been examined. The kinetic energy studies focus on drift cell parameters, including electric field strength and helium pressure. The internal energy studies focus on the effects of ionization parameters in the glow discharge. Our results indicate that the drift cell gas pressure has a large influence on the kinetic energy of these heavy ions. The data also suggest the presence of some degree of electronic excitation of the ion by the discharge.

Overpressure Layer Chromatography for the Separation of Aromatic Hydrocarbons

Robert Comer and William Holman
Faculty Mentor: Dr. R. Cameron Dorey

Overpressure layer chromatography is a thin layer chromatography technique which uses a membrane pressed onto the chromatographic stationary phase and a pumped mobile phase. This is in contrast to standard thin-layer chromatography, which utilizes capillary action to draw the mobile phase through the stationary phase in an open tank. In our system, a sample of two aromatic compounds is placed on a polar silica gel plate, and a nonpolar mobile phase (hexane) is used to carry the individual compounds across the silica gel. The polarizabilities of the compounds determine their relative affinities for the hexane and silica gel, and thus the amount of travel down the plate. The compounds then separate in space as they travel with the mobile phase, as the interactions with silica gel retard their movement. Overpressure layer chromatography reduces the spreading of the compounds as they travel down the silica gel bed, compared to regular thin layer chromatography, and thus increases the confidence in identification and lowers the limit of detection.

Supercritical Fluid Chromatography on Alumina Columns in the Separation of Aromatic Compounds

T.J. Couch
Faculty Mentor: Dr. R. Cameron Dorey

We have used supercritical fluid chromatography with carbon dioxide as mobile phase and alumina as stationary phase to separate aromatic compounds. An ultraviolet chromatography detector with a high-pressure cell built for liquids was used for detection and quantification of the compounds as they elute.

The test mixture used was naphthalene and biphenyl in carbon tetrachloride (non-retained peak). The compounds were separated on the basis of their affinity for the alumina and solubility in supercritical carbon dioxide. The polarizability of the compounds determine both the alumina affinity and carbon dioxide solubility, in inverse relation. The temperature and pressure of the carbon dioxide also strongly affect the solubilizing power of the mobile phase for all compounds by changing its density. Higher carbon dioxide densities result in lower retention times for the test compounds, and generally poorer separations.

Adjusting the carbon dioxide pressure to 2500 psi and temperature to 120 C effected a separation of the test compounds. Higher temperatures or lower pressures lengthen the time needed for a chromatographic run, without great increases in separation efficiency.

Sulfur-Bound Cysteine(Ethyl Ester) Adducts of Nickel with a Single Tris(Pyrazolyl)Borate Ligand

Russell W. Cutts, Dale Rimmer, Michael P. Ellis
Faculty Mentor: Dr. Patrick J. Desrochers

Bulky tris(pyrazolyl)borates(L-) allow the formation of four and five coordinate nickel centers(Trofimenko, S. Chem. Rev. **1993**, 93, 943). Such open nickel centers are relevant to studies of the active sites in nikcel-hydrogenase enzymes. We have used the ligand, hydrotris(3-phenyl-5-methylpyrazoly)borate(L_p^-) to prepare complexes of the form: L_pNiX ($X=CL^-$, F^-, and a variety of thiolates S^-). Visible spectra show a characteristic sulfur to nickel charge transfer that increases in energy with increase in thiolate basicity. Visible spectra also show the nickel affinity for the sulfur group over other functionalities in cysteine. Mass spectroscopy of the adduct indicates that the product is L_pNi-Cysteine Ethyl Ester. Steric bulk from the phenyl substituents of L_p^- prevent more than a single thiolate ligand from bidding to the nickel center, even when excess thiolate is present. Implimations of sulfur-bound cysteine(ethyl ester) and tris(pyrazoly)borate adducts of nickel as potential hydrogenase active-site models are discussed.

Chemical Ionization Mass Spectrometry at High Kinetic Energy in the Quadrupole Ion Trap

Sheri L. Dobbs
Faculty Mentor: Dr. R. Cameron Dorey

Chemical ionization mass spectrometry utilizes the ability of a pronated molecule to transfer a proton to a neutral (analyte) molecule. Using ammonium ion as the chemical ionization reagent provides selectivity in an analysis, since the ion will only transfer a proton to molecules of higher proton affinity, such as amines. Other substances which are in the mass spectrometer at the same time are not ionized, and contribute nothing to the mass spectrum. The great majority of the resulting ions remain intact, with a mass of $(M+1)^+$, M being the mass of the neutral analyte.

In an ion trap mass spectrometer, chemical ionization reagent ions have much higher kinetic energies than in traditional mass spectrometer sources, and some of this kinetic energy can be transferred to the analyte ion upon the proton transfer. The transferred excess energy can result in the breakage of weak bonds in the newly-formed ion, which produces fragment ions containing structural information about the original molecule. This is significant, since other common ionization methods which give structural information, such as direct electron ionization, are non selective in nature and will thus produce erroneous spectra if other substances are present in the mass spectrometer with the analyte.

X-ray Fluorescence Analysis of Meteorites

Donnie R. Golden
Faculty Mentor: Dr. Michael W. Rapp

X-ray fluorescence (XRF) is a nondestructive method of analysis used to determine the presence of various elements in a sample. Samples are irradiated with low energy x-rays and give off secondary x-rays characteristic of the elements contained in the sample. The instrument detects these fluorescent x-rays and separates them according to their intensities. The amount of each element is then identified by the intensity of its fluorescent radiation.

Since XRF is a nondestructive method, it is useful in the analysis of space related materials, specifically meteorites. Meteorites contain varying concentrations of elements which can be used to classify them. Many of these elements can be detected through the use of XRF. Standards containing known concentrations of various elements were used to prepare a calibration plot for each element. The linear proportionality was obtained for a plot of concentration of nickel versus intensity of its XRF peak. Measured intensities of the nickel XRF peak from meteorites, when compared to these calibration plots, gave good to excellent agreement with reported percentages of the elements present.

Protocol for the Purification of Histone Proteins

Jennifer Havens
Faculty Mentor: Dr. Patricia H. Draves

Over the past two and half years we have been preparing to observe antitumor drug interactions with DNA and chromatin. To do so we have focused our research on two basic areas: histone protein and DNA purification.

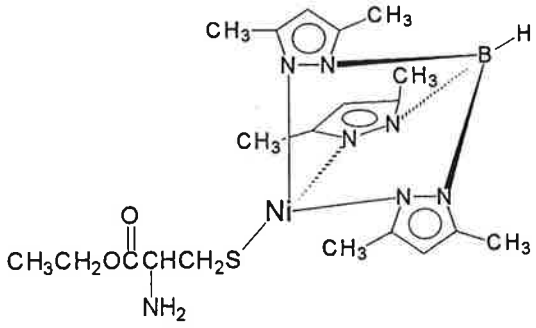
The protocol for histone purification concentrates on the gradual degradation of a chromatin complex. This was completed in three generalized steps using chicken blood as the starting material. Initially, erythrocytes were isolated from blood by centrifugation, and nuclei were released from the erythrocytes by lysis. Subsequently, chromatin was removed from the nuclei and digested with an endonuclease releasing small chromatin subunits consisting of DNA and histone proteins. Finally, a hydroxylapatite protein purification column was developed to isolate the histone proteins from the DNA. The purity of the histone proteins was monitored by SDS-polyacrylamide electrophoresis.

Future work includes the complexing of purified histones with defined sequence DNA to observe the interactions of chromatin-antitumor drug binding mechanisms and specificity of the drug for DNA in it's native confirmation.

Nickel-Hydrogenase Models: Thiolate Adducts with a Less Stercially Demanding Nitrogen Donor

Phillip K. Rice and Alex Lovelace-Chandler
Faculty Mentor: Dr. Patrick J. Desrochers

Hydrogenases are metalloenzymes that catalyze the reaction $H_2 \rightleftharpoons 2\ H^+ + 2\ e^-$. Interest in hydrogenases results from their potential use in the production of hydrogen gas from water. Hydrogen has important industrial applications and has attracted recent interest as an alternative fuel source for the future. Presented here are studies of the interactions between nickel and bound ligands such as L_M and cysteine (ethyl ester). This system is a relevant model of the active site of nickel-containing hydrogenase enzymes. L_MNiSCysEt, a green solid, was prepared under anaerobic conditions, allowing for its first reported synthesis. The compound was characterized by infrared, ultraviolet-visible, and mass spectroscopies. The ultraviolet-visible spectrum shows a characteristic sulfur-to-nickel charge transfer (S-Ni CT) peak, consistent with S-Ni CT trends observed for compounds of the formula L_M XSCysEt (X=Co,Cu). L_MNi SCysEt also shows interesting



L_MNi-SCysEt

reactivity towards molecular oxygen. Similar reactivity behavior has been reported for several nickel hydrogenases in the literature. This work will contribute to an improved understanding of the effect of oxygen on nickel hydrogenase active sites.

DNA Purification for Studies of Mitomycin C-DNA Interactions

Jason Skinner
Faculty Mentor: Dr. Patricia H. Draves

Interactions between DNA and the antitumor, antibiotic, mitomycin C, are currently being examined and characterized using restriction fragments of DNA. A protocol has been developed to purify *Lytechinus variegatus* 5S r DNA from plasmids cloned in *Eschrichia coli*. The 5S r DNA, consisting of twelve repeating sequences, has been cleaved into twelve identical units each consisting of 208 base pairs. Modifications are being made to existing protocols to increase the yields and quality of DNA. The purified DNA will be used in studies examining mitomycin C - DNA interactions by footprinting techniques. Information from this study will contribute to our understanding of antitumor drugs and their role in cancer therapy.

Optical Measurements of the ozone precursor Isoprene

Neal Yowell Faculty Mentor: Dr. Jeff Draves

Laboratory measurements of the pressure broadened absorption cross-sections of isoprene in the 10 micron region of the electromagnetic spectrum have been made. Absorption cross-sections for a Q-branch type feature centered near 985 cm^{-1} are on the order of 3×10^{-3} ppm^{-1} m^{-1}. The strength of this absorption feature indicates that a CO_2-Differential Absorption Spectrometer will have a method detection limit on the order of 2 ppb-V for a path length of 1 km. Further investigations in the 885 cm^{-1} region yield an estimated 0.6 ppb-V detection limit for the same 1 km path length. The measurements conducted show that the CO_2-Differential Absorption Spectrometer will be capable of sensitive, accurate, and rapid detection of isoprene in an *in situ* capacity.

Department of Physics and Astronomy

The Investigation of Meteorites using X-Ray Fluorescence

Christopher P. Sheesley
Faculty Mentor: Dr. Rahul Mehta

We explored the use of X-ray fluorescence (XRF) in determining the composition and classification of meteoritic samples. Radioactive sources of ^{55}Fe, ^{244}Cm, ^{241}Am were used to fluoresce the meteorite samples. A LN_2 cooled ORTEC Si(Li) detector with a 0.3mil Be window was used to detect the photons fluoresced from the sample. The data was collected and analyzed by a PCA III data acquisition card installed in a pentium computer. Efficiency of the detector was determined by (i) measuring calibrated radioactive sources and (ii) determining the attenuation of photons through the various layers. The ^{55}Fe source was found to be the best source to detect the silicates present in stony meteorites. While the ^{244}Cm was found to be the best at detecting iron and nickel in the iron meteorites. The program Quantum was used to strip and smooth the resulting spectra. Determining the best method of detecting elements present in meteorite samples was the main focus of this research project.