BIOCHEMISTRY

Nucleoside analogues as antiviral drugs
SERINA A. Aubrecht (Dr. Abul Fazal and Dr. Henry Jakubowski, Chemistry)

Nucleoside analogues are a group of antiviral chemical compounds that are similar in structure to nucleosides (the backbone elements of DNA and RNA), but different in respect to elemental composition by the replacement of a hydroxyl (OH) group by a hydrogen (H) on their ribose sugars. They are used to prevent viral replication in infected cells and are found in chemotherapy and HIV drugs. This study analyzes the mechanisms in which these antiviral drugs function.

Lipopolysaccharide stimulated phosphorylation of macrophage MAP kinases ERK1 and ERK2.
KERRY BAUER, DUSTIN BENTLEY, KEVIN BETTENDORF, KATHERINE HARTJES, KATHLEEN HROMATKA, and SAMANTHA VANWECHEL (Dr. Barbara May, Biology, and Dr. Henry Jakubowski, Chemistry)

Lipopolysaccharide, a common molecule on the surface of gram negative bacteria, was used to stimulate mouse macrophage cells, components of the innate immune system. This activation initiates a signal transduction pathway, characterized by phosphorylation of proteins. The macrophage cells will be lysed and the phosphorylated proteins will be isolated, separated, and identified by 2D polyacrylamide electrophoresis, Western blot, and mass spectral analyses. The techniques and procedures developed during this project could be used in future research into other phosphorylated proteins that are found to be prevalent in certain cancers such as breast cancer.

Purification and analysis of wild type and mutant low molecular weight protein tyrosyl phosphatases.
ROBERT HLAVACEK
(Dr. Henry Jakubowski, Chemistry).

Wild type (WT) and mutant low molecular weight protein tyrosyl phosphatases (LMW-PTP) were expressed in E. coli as fusion proteins with glutathione-S-transferase (GST). Successful purification of the fusion proteins and the LMW-PTP separated from GST by selective proteolysis was accomplished using GST-affinity chromatography. Purification was monitored using polyacrylamide gel electrophoresis (PAGE), measuring absorbance at 280 nm, and monitoring LMW-PTP enzymatic activity spectrophotometrically through the cleavage of p-nitrophenyl phosphate (pNPP) at 405 nm. Enzyme kinetic analyses of pNPP cleavage by WT and active mutants (W39F and W49F) confirmed that inorganic phosphate acts as a competitive inhibitor. Circular dichroism spectroscopy indicated that the inactive C12S mutants (W39FC12S and
W49FC12S) have secondary structure characteristic of the WT LMW-PTP. Fluorescence quenching of the PTP W39F mutant by inorganic phosphate, phosphotyrosine, pyridoxal phosphate, and phospho-peptides is being explored as a method to characterize inhibitor binding.

**Wilson's disease: analysis from a biochemical approach.** KATHLEEN HROMATKA (Dr. Dave Mitchell, Biology)

Wilson’s disease is a rare but serious condition characterized by the accumulation of copper in the liver. Genetic mutations of the ATP-ase-7B liver enzyme result in the enzyme’s inability to function normally in its role of preparing dietary copper for expulsion from the body. The resultant accumulation of copper leads to a variety of symptoms that, if left undiagnosed and untreated, are fatal. Recent research has provided new insight into the mechanism of action behind the affected enzyme.

**The synthesis and purification of fluorescein-labeled phosphotyrosine and the study of its interaction with protein tyrosyl phosphatase using spectrofluorometric anisotropy measurements.** BENJAMIN M. MURRAY (Dr. Henry Jakubowski, Chemistry).

The purpose of this research is to synthesize an inhibitor of protein tyrosyl phosphatase, fluorescein-phosphotyrosine (FPY). Phosphotyrosine (PY) was labeled with fluorescein isothiocyanate and purified using reverse-phase HPLC on a preparative C-18 column eluted with an acetonitrile gradient in an ethanolamine buffer (pH 9). The final products of the synthesis were characterized and analyzed for purity using LC-MS. The purified FPY will be used to obtain spectrofluorometric anisotropy measurements to study the binding properties of FPY to protein tyrosyl phosphatase.

**Quantitative real-time polymerase chain reaction (qRT-PCR) and its use in determining GAL 1 and GAL 10 levels in *Saccharomyces cerevisiae*.** BETH NOMELAND and EMILY HEMANN (Dr. Michael Reagan, Biology).

The research goal was to develop a qRT-PCR protocol for the quantification of the galactose-induced GAL1 and GAL10 genes in *Saccharomyces cerevisiae* using purchased primers ordered for the GAL1, GAL10 and 18S genes. The 18S gene is a ribosomal RNA gene and was used as a control to normalize calculated results. The *S. cerevisiae* cell cultures were grown in various media after which the mRNA was isolated using YeaStar RNA kit from Zymo Research. The qRT-PCR reaction was then set up using Qiagen Quantitect SYBR Green RT-PCR Kit and the Qiagen Mini-Opticon thermal cycler to quantify gene expression using fluorescence. Primer concentration optimization was needed to ensure accurate amplification results. Results will be discussed as well as uses of the method to further investigate gene transcription in future research projects.
The fountain of youth: a biochemical explanation of physiological aging from environmental and endogenous oxidative stressors. ZACHARY R. SHAHEEN (Dr. David Mitchell, Biology).

The presence of reactive oxygen species, whether from environmental or endogenous sources, correlates with decreased organismal longevity and increased risks of neurodegenerative and cardiovascular diseases. Reactive oxygen species are most typically formed from metabolic activities, antibody-catalyzed ozone formation in the body, or from exogenous environmental pollutants. This typically reacts with protein residues, lipids, and DNA to form inactive proteins, lipid peroxidation, and nucleic acid structural damages. Reactive oxygen species initiate important cellular stress signaling pathways through growth and metabolic regulation, and modulate transcription factors important in cellular repair or apoptotic capabilities. Current studies look to decrease oxidative stress through caloric restriction, antioxidant dietary supplementation, or other novel therapeutic techniques.

Crystallization of ERK-1 and RSK-2 protein complex. VALERIE M. STEINMAN (Dr. Henry Jakubowski, Chemistry).

The study of protein structure is important to help define and understand protein function. The function of the RSK-2 protein is of particular interest as studies have shown mutations can cause cancer. The ERK-1 protein is known to bind with the RSK-2 protein to activate the RSK-2 protein. We attempted to crystallize the RSK-2 and ERK-1 complex for use in X-ray structure analysis. Various domains of the proteins ERK-1 and RSK-2 were purified and combined together with different well solutions to test for optimal conditions for crystallization. Once optimal crystals are produced, then with further analysis, the changes in the original RSK-2 structure could be evaluated to understand the activation of the RSK-2 protein.

Detection and mechanism of action for common anti-anxiety pharmaceuticals
TYLER J. THORSON (Dr. Alison Johnson, Chemistry)

Many different drugs have been discovered for the treatment of anxiety and depression disorders. These drugs aim to counteract the chemical imbalance that is present in patients with these illnesses by regulating brain levels of serotonin, norepinephrine, dopamine, and other chemicals. To do this, researchers have developed many different techniques, such as Monoamine oxidase (MAO) inhibition, serotonin reuptake inhibition, and tricyclic anhydrides. Studies have been done on each form of anti-anxiety medication in an attempt to determine the mode of action at the synapse between pre- and post-synaptic membranes. Such mechanisms include enzymatic action, physical blockage of reuptake channels, and chemical blockage of channels. Each drug targets a specific brain chemical via a specific mechanism.
The role of iron in Parkinson’s disease.
MARIE F. ZETTEL (Dr. Brian Johnson, Chemistry).

Parkinson’s disease one of the largest neurodegenerative diseases today. The causes are not well understood; however there is a high correlation between regional iron buildup and Parkinson’s disease. The role of iron in Parkinson’s has been unknown until recently. Scientists have been able to show that excess brain iron reacts with hydrogen peroxides causing oxidative stress and cell death. Two possible explanations for buildup of iron in the brain are protein misregulation and problems with the brain-blood barrier. In order to decrease destructive iron levels, chemists are developing molecules that would remove harmful iron from the brain and stop neurodegeneration.

CHEMISTRY

The synthesis and study of multi-copper oxidase active site models: structural and functional biomimetic complexes.
JEFFREY S. BANDAR (Dr. Brian Johnson and Dr. T. Nicholas Jones, Chemistry).

Tricopper oxidase enzymes, such as laccase, ascorbate oxidase and ceruloplasmin, couple the four electron reduction of oxygen to the oxidation of substrate molecules. The mechanism of action of these enzymes is not well understood. In order to explore this class of enzymes, several copper complexes were prepared and studied. Initially, it was determined that a complex containing pyridyl copper binding sites degraded while reacting with oxygen. These results motivated the recent synthesis of a triazole containing ligand, synthesized utilizing “click chemistry” methodology. The complete syntheses, characterizations and reactivity studies of these complexes are presented in this study.

Isolation of kumepaloxane from marine natural products. SARAH DeMARAIS (Dr. Kate Graham, Chemistry).

Researchers have found a great interest in the isolation of marine natural products. One in particular is the compound kumepaloxane that is found in the mucus and digestive glands of the brightly colored bubble shell called *Haminoea cymbalum*. The bubble shell excretes kumepaloxane through its mucus that is used as chemical defense against carnivorous fish. The bubble shell was collected from a reef flat in Pago Bay, Guam. The isolation, structure, stereochemistry, and conformation of kumepaloxane were analyzed.

The photodecomposition of the antidepressant pharmaceutical drug venlafaxine (Effexor) in natural sunlight. BENJAMIN P. KRAGE (Dr. Michael Ross, Chemistry).

Venlafaxine (trade named Effexor) has been recently found as a new form of pollutant near wastewater treatment plants due to the inability to remove the chemical through current treatment processes. Experiments were run in simulated surface water conditions to determine venlafaxine’s
rate of decomposition in natural sunlight and to isolate any stable decomposition products. By
means of HPLC analysis, the half-life of this decomposition process was determined to be
approximately 40 hours at pH 3 and on the order of several hundred hours at pH 5 and pH 9. A
series of stable decomposition products were also found to be formed. Due to the extended period
of time required for decomposition, venlafaxine could prove harmful to aquatic environments.

Synthesis of two ligands to model the active site of multicopper oxidase. BRADLEY
MCGUIRE (Dr. Brian Johnson, Chemistry).

Two ligands were to be synthesized in hopes that one would be a perfect model of the active site for
multicopper oxidase which would allow for the study of the reaction mechanism. One ligand
contained an extra carbon in the spacer arm which would allow for greater flexibility. The other
ligand would contain methoxy arms instead of the standard ethyl arms which would eliminate the
problem of ethyl migration. Synthesis of these two pure ligands is still in progress; as a result it is
unknown whether or not either ligand is an acceptable model for the active site of multicopper
oxidase.

Mapping the magnetic domains of Fe₃O₄ nanopatterns on mica (0001) using a newly
developed atomic force microscopic mode: magnetic sample modulation. HA H. PHAM
(Dr. Jayne C. Garno, Chemistry, Louisiana State University, and Dr. Chris Schaller, Chemistry).

Magnetic sample modulation (MSM) is a newly-developed atomic force microscopy (AFM) imaging
mode used to detect and map magnetic properties of nanoparticles on surfaces. The principle of
MSM is based on using an AFM tip to detect the vibrational response of magnetic nanomaterials
with the application of an external electromagnetic field. For MSM, a soft non-magnetic tip is kept
in contact with the sample surface during scanning to obtain high resolution. The magnetic domains
on a surface are interpreted in the resulting MSM phase and amplitude images. To test the
applicability of MSM, nanopatterns of magnetite nanoparticles were generated on mica via two-
particle lithography. Successful results in detecting Fe₃O₄ nanoparticles suggest a potential impact of
MSM for interdisciplinary areas such as biomedicine, nanotechnology and material sciences.

Progress in the total synthesis of 6-methyl pacifigorgiol. SAMANTHA VANWECHEL (Dr.
T. Nicholas Jones, Chemistry).

Pacifigorgiol is a marine natural product isolated from the sea fan *Pacifigorgia* cf. *adansil*. The structure
was determined by W. Fenical and was synthesized by M.G. Martin at Cornell University. The
purpose of synthesizing the 6-methyl derivative is to examine this synthetic route and for use in
further study of the mechanism of biological activity of pacifigorgiol. We have successfully
completed the first step, the formation of the epoxide. The second step is to open the epoxide with
a higher order cuprate reaction. The side synthesis of a reactant to be used in the second step is our
current focus.