

Undergraduate Oral Presentations II

Organizer: John G. Kaup Clemson University, Clemson, SC

724. Investigation of 5,10-Methenyltetrahydrofolate Synthetase

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5,10-methenyltetrahydrofolate synthetase (MTHFS) is an enzyme that catalyzes the conversion of 5-formyltetrahydrofolate (folinic acid) into 5,10-methenyltetrahydrofolate along with the hydrolysis of ATP. While folinic acid does not directly participate as a cofactor in metabolism, the reaction catalyzed by MTHFS and subsequent reactions convert it into cofactors that are essential to many cellular processes. These include pathways involving DNA and ATP synthesis, DNA repair, and protein synthesis. The purpose of this research is to better understand the mechanism by which MTHFS catalyzes the conversion of folinic acid through an investigation of which amino acids in the enzyme contact the substrates. To accomplish this, individual amino acids in the protein are changed through site-directed mutagenesis to alanine. The resulting mutant protein's affinity for its substrates is then characterized through kinetic assays. The role of lysine at position 3 of the protein will be discussed in regards to the structure and function of this important enzyme.

725. Extraction of Phenolic Compounds from Soy Hull

Latoya Elizabeth Whitley and Uruthira Kalapathy, Claflin University, Orangeburg, SC

Polyphenolic compounds are present in various edible plant sources and confer antioxidative properties making them nutritionally valuable agents against cancer and arteriosclerosis. This study was aimed at investigating the various effects of extraction temperature, extraction time and other variables intrinsic to the extraction process, on the yield of phenolic compounds from Soy hull. The phenolic content of Soy hull extracts were also quantitatively compared to that of notable sources such as green tea and grape skin. Total phenolic content was determined by the Folin-Ciocalteu method which involved UV-vis spectrophotometry and a calibration standard curve based on catechin standards. For comparative analysis of the soy hull, grape skin and green tea extracts, the extractions were all conducted using a 1:5 ratio of solute to 70% ethanol solvent and mixed for 1 hr at room temperature. The total phenolic contents of the green tea, grape skin and Soy hull extracts expressed as catechin equivalents (mg of catechin/ g of sample) were, 153.39 mg/g, 4.527 mg/g and 0.623 mg/g respectively. For Soy hull, increasing extraction temperature to 70C improved extraction efficiency, with a yield of 0.684 mg/g. At 80C, there was no marked increase in extraction efficiency which was lower than what was expected. This may be due to the presence of a rigid pectin framework. A preliminary study in our laboratory indicated that an acid digestion prior to extraction may improve phenolic compound extraction yield. The combined effect of temperature, acid treatment and extraction time on phenolic compound extraction will be reported.

726. Developing Nanoscale Colloid Displacement Lithography

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We have expanded gold colloid particle manipulation techniques using a scanned probe microscope into a nanolithography platform. We have utilized 5- and 10-nm gold colloid particles adsorbed on poly(diallyldimethylammonium chloride) to consistently pattern and create uniform features on the order of 200 nm and smaller. The gold colloid particles were manipulated with a silicon cantilever having a force constant of about 3 N/m and a resonant frequency of approximately 70 kHz. We have also achieved two-dimensional aspect ratios approaching 50:1 in repeat patterns covering 25 μm^2 . We have further employed electroless deposition to fuse the colloid particles together in anticipation of creating working interdigitated arrays. While the electroless deposition causes an increase in the final dimension of the

features, the increases is predictable enough that we are able to pattern appropriately to achieve the desired final dimensions. We will describe the methods used to accomplish these ends and discuss the challenges associated with creating intricate patterns using this technique.

727. Why Are Esters and Amides Weaker Carbon Acids Than Ketones? Contributions by Resonance and Inductive Effects

Joel M. Karty¹, **Alexandra Fersner**¹ and Yirong Mo², (1)Elon University, Elon, NC, (2)Western Michigan University, Kalamazoo, MI

Esters and amides are less acidic at the alpha carbon than ketones are. In water, methyl acetate (a simple ester) and N,N-dimethylacetamide (a simple amide) are less acidic than acetone by about 5 pKa units and 10 pKa units, respectively. In the gas phase, those numbers are 2.7 kcal/mol and 5.7 kcal/mol. Organic chemistry textbooks typically attribute these differences in acidity to resonance effects alone. However, the RO and R₂N groups in esters and amides, respectively, are electron-withdrawing, so inductive effects play a significant role as well. Because such resonance and inductive effects contribute simultaneously, the acidity differences above cannot provide an accurate measure of either effect.

The goal of our research was to determine the contribution by resonance and inductive effects toward the acidity differences among ketones, esters and amides. To do so, we employed two different computational methodologies. One is a vinyllogue methodology similar to that which Holt and Karty used to determine the resonance contribution of formic acid's enhanced acidity over methanol (J. Am. Chem. Soc., 2003, 125, 2797). The second is a block-localized wavefunction methodology, developed by Mo and coworkers (J. Phys. Chem. A., in press), used to obtain the absolute energies of specific resonance structures. Our results suggest that both the RO group in an ester and the R₂N group in an amide decrease the acidity via resonance, by 3-5 kcal/mol and 3-11 kcal/mol, respectively. Inductive effects decrease the acidity by about 5 kcal/mol and <2 kcal/mol, respectively.

728. Fluoro-Lipophilic Biphasic Liposomes

Christopher J. Pollock, Jennifer S. Kauffman and William T. Pennington, Clemson University, Clemson, SC

Liposomes possess much promise for use in pharmaceutical delivery because of their ability to encapsulate molecules within an inner cavity. This isolation from the outside environment provides protection for sensitive pharmaceuticals and the ability to deliver the drug to a specific target. Liposomes in this project were prepared using dimethyldioctadecylammonium bromide ([DMDOA]Br) and potassium perfluorooctane sulfonate (K[PFOS]) in a biphasic mixture of chloroform (CHCl₃) and perfluorooctane (CF₃(CF₂)₆CF₃). In some reactions water was added to form a third phase capable of removing the KBr formed during the reaction, and this was found to be very effective. Sonication was performed on the resulting mixtures to produce biphasic DMDOA⁺PFOS⁻ liposomes possessing a lipophilic exterior and fluorophilic interior or a fluorophilic exterior and lipophilic interior. The ionic compound DMDOA⁺PFOS⁻ and the associated liposomes were characterized using light scattering (LS), powder x-ray diffraction (XRD), Fourier Transform infrared spectroscopy (FTIR), and nuclear magnetic resonance (NMR).

729. Aggregation Kinetics and Thermodynamics of SWCNTs Due to Ruthenium Coordination Complex Interactions

Andrea N. Giordano, University of North Carolina at Charlotte, Charlotte, NC

Single Walled Carbon Nanotubes (SWCNTs) show effective binding with varying ruthenium complexes in N, N-Dimethylformamide (DMF). Enantiomerically pure ruthenium metallodendrimer [Λ6E3Ä-Ru10]20+[PF6-]20 (Ru(dec)) is shown to bind strongly and specifically to the SWCNTs. Intensive studies show that at varying concentrations of coagulants, the stable dispersions of carbon nanotubes aggregate

and begin to flocculate. The critical coagulation concentrations (CCC) have been determined for +1, +2, and +3 inorganic salts, the +2 Ru(phen)₃, and the +20 ruthenium metallodecamer coagulants. We studied the binding capacity and binding kinetics with UV-VIS spectroscopy, Atomic Absorption spectroscopy, dynamic light scattering and zeta-potential measurements. Future research areas of interest are the CCC of varying ruthenium oligomers and the effect of the water on the CCC for varying coagulants. Analysis of current theoretical models is given with regard to these experimental data. Potential applications toward 3-dimensional nano-manufacturing will be discussed.

730. Developing Analogs of the Antibiotic Cytosporone E

Elizabeth Hunter Flynn, Meg Callanan and Dr. Justin K. Wyatt, College of Charleston, Charleston, SC
The antibiotic cytosporone E, isolated in 2000 and found to have weak antibiotic activity, has recently been found to only display activity against gram-positive bacteria. The apparent "business end" of the molecule contains three phenolic moieties, of which the central moiety is need for antibiotic activity. What is the role of this central hydroxy group? Does it necessarily have a specific function, or does the molecule simply need something in this position to take up space? To help determine its role a derivative will synthesized, where a vinyl group will take the place of the central hydroxy group. This vinyl group will be replaced with different substituents with varying properties that will help in the development of a structure activity relationship (SAR) study to hopefully improve the antibiosis of the antibiotic.

731. Metal Specificity of the Ribonucleotide Reductase from *Coryneform Ammoniogenes*

Amy Rhoden, J. Ryan Yonce and Pamela Riggs-Gelasco, College of Charleston, Charleston, SC
The metalloenzyme ribonucleotide reductase (RNR) catalyzes the conversion of ribonucleotides to deoxyribonucleotides. The enzyme's prominent role in DNA synthesis makes it an ideal anticancer, antiviral and antibacterial drug target. The RNR of higher organisms utilizes the reactivity of a Fe(II)/Fe(II) cluster with oxygen to oxidize a tyrosine residue to a tyrosyl radical and a diferric cluster cofactor. Research in our laboratory suggests that *Coryneform ammoniogenes* may be capable of utilizing a manganese cofactor in its RNR to catalyze the same reaction, despite its gene sequence similarity to Fe RNRs. Our objective is to define the metal specificity of the RNR from *C. ammoniogenes*. Differences in the oxygen-dependent cofactor chemistry among the RNRs could potentially be exploited for drug development. Our work is focused on optimizing conditions to generate the active radical/metal cofactor in CA-RNR. Here we report our spectroscopic characterization of the RNR cofactor from *C. ammoniogenes* and our progress in isolating *nrdI*, a protein that may be responsible for enhancing RNR activity in some bacteria.

732. Simultaneous Extraction of Pectins and Phenol Compounds from Soyhull

Rachael Woods and Uruthira Kalapathy, Claflin University, Orangeburg, SC
Pectin is a major structural component of plant cell walls. Therefore, the efficiency of extraction method involving seed coats, hulls and other relatively rigid parts of plant materials might be affected by the presence of pectin. The objective of this study was then to determine the effect of simultaneous extraction of pectins and phenolic compounds from soy hull on extraction efficiency. Soy hull pectins were extracted by acid digestion at 70, and 90oC followed by precipitation with isopropanol for 24 -36 hrs. The pectin precipitates were separated by centrifugation and the supernatants were analyzed for phenolic compound content based on a standard catechin calibration. The pectin precipitates were washed with 50% isopropanol/1 M hydrochloric acid, dried and confirmed by FTIR spectrometry. Based on preliminary data, the total phenolic content of the extract after pectin removal at 70 and 90oC, expressed as catechin equivalents were 0.64 and 1.5 mg/g of soy hull, respectively. The early data indicated that simultaneous pectin and phenolic compound extraction efficiency maybe temperature dependant. The

amount of pectins recovered from soy hull extract at 70°C was about 40mg/g of soy hull. Studies will be directed to confirm these results and establish an optimal condition for the extraction of pectins and phenolic compounds.

733. Binding Interactions Elucidate Function of Yeast Transcription Factor Iws1

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Iws1 is an essential transcription factor lacking enzymatic activity in the yeast *Saccharomyces cerevisiae*. Its human ortholog has been shown to bind with Spt6, a transcription factor which binds to the carboxyl-terminal domain of elongating RNA Polymerase II, and functions in 3' mRNA processing through that interaction. Interactions between human Iws1 and REF1/Aly (Yra1 in yeast), an RNA export factor, have also been demonstrated, and it is believed that human Iws1 aids in recruiting REF1/Aly to Spt6-dependent genes. In this study, His-tagged recombinant yeast Iws1 is overexpressed in strains of *Escherichia coli* and isolated and purified through immobilized metal ion chromatography. The pure Iws1 is then used in far-western protein binding assays to determine its binding interactions in vitro and clarify its function in transcription. These binding experiments are used to test for association between Iws1 and Yra1 and confirm the location of that interaction on Yra1. Resulting data show Iws1 to associate with the carboxyl terminal end of Yra1 with good specificity. The binding patterns of Iws1 provide insight into the machinery of transcription and mRNA export in yeast.