1570-Pos Ethanol Suppresses Respiration Stimulated by Ureagenic Substrates in Cultured Rat Hepatocytes: Partial Reversal by Inhibition of Alcohol Dehydrogenase and Cytochrome P450 but not by Acetaldehyde Dehydrogenase

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BACKGROUND. Ethanol is a hepatotoxicant to humans that alters hepatic metabolism. Ethanol undergoes 2-step oxidation to acetaldyhyde in the cytosol by alcohol dehydrogenase (ADH) and cytochrome P450 2E1 (2E1) and then to acetate by acetaldehyde dehydrogenase (ACDH) in mitochondria. Previous studies showed that ethanol suppressed mitochondrial metabolism in permeabilized hepatocytes (*BBA* **1762**, 181). The goal of this study was to determine the effect of ethanol metabolism on mitochondrial respiration in intact hepatocytes.

METHODS: After 18 h in culture, rat hepatocytes in Krebs-Ringer-HEPES buffer were treated with ethanol (0–100 mM) and ureagenic substrates (in mM: 3 NH_4Cl, 5 L-ornithine, 5 Na-lactate) in the presence and absence of cyanamide (200 μM, 2E1 inhibitor), 4-methylpyrazole (4-MP, 100 μM, ADH inhibitor) or aminobenzotriazole (ABT, 1 mM, ACDH inhibitor). Oxygen consumption rate (OCR) was measured with a Seahorse XF24 analyzer.

RESULTS: Ureagenic substrates doubled OCR with an initial 25% stimulation within 5 min followed by a progressive increase over the next 45 min. Ethanol dose-dependently suppressed OCR stimulated by ureagenesis with half maximal inhibition at ~50 mM. At 100 mM, ethanol suppressed stimulated OCR by >80%. In the presence of 100 mM ethanol, cyanamide and 4-MP restored stimulated OCR by 18 and 26% respectively and in combination restored OCR by 36%. ABT did not prevent suppression of stimulated OCR by ethanol.

CONCLUSIONS: ATP demand created by ureagenesis stimulates respiration by cultured hepatocytes. Ethanol suppresses this stimulation, an effect partially reversed by ADH and 2E1 inhibitors but not by inhibition of ACDH. Thus, ethanol-dependent suppression of mitochondrial respiration may be the consequence, at least in part, of cytosolic generation of acetaldehyde and/or NADH.

X-Ray Diffraction

1571-Pos Hybrid Methods In The Structure Determination Of Extremely Large Proteins

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Gastropod hemocyanins are extremely large oxygen transport proteins (8MDa), which cooperatively bind oxygen at type-3 copper centers. Native hemocyanin is a cylindrical didecamer made up from a subunit (400kDa), which contains 7 homologous functional units (FU, 50kDa) and one FU (FU-h) with an additional tail of ~100aa. While the structure of two FUs is known, the spatial arrangement of the FUs within the didecamer and the fold of the FU-h tail remain unclear. We use a hybrid method, i.e. 3D-cryo-electron microscopy and X-ray crystallography, to obtain structural information.

The enormous size of hemocyanin makes protein crystallography daunting but not impossible. We grew crystals of a cephalopod hemocyanin (3.5MDa, *Octopus*), which represents one half of a gastropod hemocyanin. These crystals diffracted to a resolution of 4Å at room temperature. Due to their radiation sensitivity cryo conditions were used for data collection, which as a side effect reduced the diffraction limit to 7Å. The dataset shows a small peak in the self-rotation function at ξ=72°, as expected from the fivefold symmetry of hemocyanin. Using this peak to fix the orientation of the five-fold axis, we wish to use molecular replacement with low-resolution models to obtain starting phases for these data.

Conversely, X-ray structures may be placed in the 3D-density map obtained from cryo-EM experiments. FU-h from keyhole limpet hemocyanin was crystallized and a 4Å dataset was collected, which was phased with molecular replacement. The major part of FU-h has the canonical fold of a molluscan hemocyanin FU, but surprisingly the C-terminal tail displays a typical cupredoxin fold. Thus, FU-h combines a type-3 copper protein fold in the N-terminal region with an unexpected type-1 copper protein fold at its C-terminus.

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1572-Pos Physical Properties of Cryoprotective Solutions for Cryo-Crystallography

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Macromolecular crystallography has undergone a major transformation in the last decade, in which most structures are now determined at cryogenic temperature in order to minimize crystal degradation from radiation damage effects. The cryogenic cooling process, however, is still accomplished mostly via trial and error. Cooling can induce crystal damage, and minimizing this damage is still somewhat of an art form. One way of minimizing cooling-induced damage is to supplement the crystal storage solution with cryoprotective molecules, called cryoprotectants. Many different cryoprotectants have been used for cryo-crystallography, although glycerol is probably the most common. There are, however, few general principles available to guide researchers in deciding on the makeup of the cryoprotective solution. To help fill this gap, we present the results of our efforts to characterize the physical properties of cryoprotective solutions. We measured the contraction of binary solutions (50 % w/w) of cryoprotectant and water when rapidly cooled from room temperature into

liquid nitrogen at 77K. Twenty different solutions were measured, resulting in a range of contraction between 0 and 15%, depending on the identity of the cryoprotectant. We compare these results to literature values for room temperature thermal contraction, and find a reasonable correlation. We also compare these thermal contraction data to literature values for other bulk physical properties important for cryocooling, including density and viscosity. We discuss how knowledge of these physical properties can be used to make more informed choices during the optimization process of cryocooling for cryo-crystallography.

1573-Pos Automated SAXS Measurements of Protein Solutions with a Laboratory Based SAXS System

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Combining the availability of a high-flux laboratory small-angle X-ray scattering (SAXS) camera employing a high-brilliance microbeam delivery system with point-focus and automatized data evaluation software, we are developing a compact and reliable system for online and high-throughput measurements for low resolution structures of proteins in solution. During such automated SAXS measurements the radius of gyration and relative mol.wt., as well as the real-space function (distance distribution function) of the scattering curve (after buffer subtraction) are calculated in certain time-intervals and the measurements are automatically stopped if no significant changes or improvements in the real-space functions can be achieved. Additionally, a low-resolution model is calculated. First results, using the protein lysozyme as a benchmark test, will be shown.

1574-Pos Structural Studies Of Diterpenes From Ageratina Vacciniaefolia

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Using Nuclear Magnetic Resonance and monocrystal x-rays Diffraction techniques we have determined the structural formula and the three-dimensional configuration of two substances extracted from the Ageratina Vaciniaefolia , a native Colombian paramus plant. The plant was collected in paramus named Cruz Verde which is located on the route Bogotá - Choachí. It was put under a process of extraction using solvents with increasing polarity, and the substances were isolated by means of Column Chromatography and fine layer.

The identification of the substances was made using nuclear magnetic resonance spectroscopy (NMR), infrared spectroscopy (IR), fusion point, optical activity and behavior of the substances before some chemical reagents. The substances Angehu2 and Angehu3 were identified like (−) - 17- (ß- glucopiranosiloxil) - kauran-19-oico-acid and (−) - 16- (ß- glucopiranosiloxil) - 17-ol-kauran-19-oico-acid.

For the determination of the three-dimensional configuration of the substances, monocrystals were obtained using the method of slow evaporation from a solution. The crystallographic parameters and the factor phases were obtained directly from the intensities of X-Rays diffraction pattern (direct methods). Model refinement was made using the method of least square.

The interest on the structural studies of these substances is centered in its possible pharmacological use.

Cryo-Electron Microscopy & Reconstruction

1575-Pos Atomic Structure Of Cytoplasmic Polyhedrosis Virus By Cryo-electron Microscopy

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Cytoplasmic polyhedrosis virus (CPV) belongs to the *Cypovirus* genus within the *Reoviridae* family. CPV is unique in having a turreted single-layer capsid encapsulated in polyhedra. Despite of its organizational simplicity, CPV is distinctively stable and fully capable of cell entry and mRNA transcription, processing and releasing. Here we report the structure of CPV at 3.88-Å resolution using single-particle cryo-electron microscopy, the highest-resolution structure so far achieved by this technology. The 3.88 Å map clearly shows the turns and deep grooves for α helices, the separation of β-sheet strands, and densities for loops and many bulky side-chains. For the first time, the map has enabled us to build *ab initio* atomic models for capsid proteins. A conformational switch has been observed between the two states of capsid shell proteins, most probably to accommodate and facilitate packing and transcription of the dsRNA genome. We discovered an mRNA releasing hole coupled with the mRNA capping machinery. A unique β-strands-rich N-terminal domain of the turret protein, has been identified, which is responsible for specific polyhedrin binding.

1576-Pos Development of a Reaction Mixer/Micro-Nebulizer for Time-Resolved Cryo-Electron Microscopy of Macromolecular Systems

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