PS04.17.13 COMPARISON OF *PNEUMOCYSTIS CARINII* DIHYDROFOLATE REDUCTASE INHIBITOR-COFACTOR TERNARY COMPLEXES. Nikolai Galitsky, Vivian Cody, Joseph R. Luft, Walter Pangborn, Hauptman-Woodward Medical Research Institute, Inc., Buffalo, NY 14203; A. Gangjee, Duquesne University, Pittsburg, PA 15282, S. F. Queener, Indiana Univ., Indianapolis, IN 46202

Pneumonia caused by opportunistic infectious agents is a major cause of mortality among patients with AIDS. Antifolates have been shown effective against the dihydrofolate reductase (DHFR) from *Pneumocystis carinii* (Pc) which is a target for drug design studies. Crystals of recombinant Pc DHFR ternary complex with the highly selective novel furopyrimidine sulfonaphthalene antifolate are monoclinic, space group $P2_1$, with lattice constants of a = 37.552, b = 43.256, c = 61.389Å and ß = 94.97° and diffract maximally to 2.1Å resolution. Inspection of difference electron density maps of the structure refined to 2.3Å resolution with R = 18% using XPLOR revealed electron density corresponding to the cofactor NADPH and the inhibitor which was incubated with the enzyme prior to crystallization. However, the cofactor and inhibitor were refined with partial occupancy as the substrate site is also occupied by folate which remained in the enzyme after purification. The largest differences between this Pc complex and those reported previously involve the conformation of NADPH and the regions encompassing residues 58-69, 81-88 and 110-115. A twist about the pyrophosphate bond places the nicotinamide-ribose ring system further from the N4 amine of this sulfonapthyl inhibitor. There is an expansion of the active site region is this structure compared to that observed for the less selective classical furopyrimidine antifolate as reflected in the shift between the alpha atoms of S58 (1.5Å) and R82 (1.7Å). Potential energy surfaces calculated with DELPHI show that the entrance to the active site of Pc DHFR has a greater positive surface than hDHFR. Also, the only negative region on this surface is that from Glu-62 of hDHFR and Glu-63 of Pc DHFR which occupy different spatial regions on the surface. Furthermore, there is a reversal in the hydrophobicity in this region resulting from sequence changes at residues 63 and 67 (E/F and L/E, for Pc and h, respectively). These results imply that this region could have an influence on inhibitior selectivity.

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PS04.17.14 PRELIMARY STUDIES OF THE N AND P PROTEIN COMPLEX FROM VESICULAR STOMATITIS VIRUS. Todd J. Green, Ming Luo, Department of Microbiology, Center for Macromolecular Crystallography,University of Alabama at Birmingham, Birmingham, AL USA

Purification and preliminary crystallization trials of the N and P protein complex from Vesicular Stomatitis Virus (VSV) are being done. VSV is a member of *Rhabdoviridae* family of viruses which are characterized by their rod-like shaped morphology. VSV is responsible for causing infections in a variety of hosts ranging from cattle to humans and as a result is a virus that has undergone much research. It has 5 genes that are encoded by the positive complement to its negative-sense RNA genome. The VSV ribonucleoprotein complex is composed of the genomic RNA in association with the N, P, and L proteins and constitutes the infectious core of the virus. N is the nucleoprotein that enwraps the 11 kilobase genomic RNA. P is a phosphoprotein and L is believed to be a part of the RNA-dependent RNA polymerase. Both are needed, along with the nucleocapsid template, for VSV transcriptase activity.

We are coexpressing the N and P proteins in an *Escherichia coli* expression system from a single plasmid. Coexpression aids in their abilty to exist in a soluble form. N is expressed with a poly-His fusion tag; while, P is expressed as the native protein. Purification is done over a Ni column and because of the association of P with N both proteins co-purify in a single step. An addition size exclusion column is used to obtain a high purity product. This purified complex is the focus of preliminary crystallization and crystallographic studies. We have obtained crystals and are optimizing these conditions.

PS04.17.15 THE STRUCTURAL NATURE OF FREE FATTY ACID TRANSPORT IN CIRCULATING PLASMA. Joseph X. Ho[1], Brian Chang[1], Kim Keeling[1], Eugene W. Holowachuk[2], Ted Peters[2], Daniel C. Carter[1], [1]NASA, ES76 Laboratory for Structural Biology,Marshall Space Flight Center, Huntsville, Alabama, USA, [2]Mary Imogene Bassett Hospital, Research Institute, Cooperstown, USA

Strutural studies of canine and human serum albumins both complexed with selected long-chain fatty acids reveal the nature of bound ligand. Novel protein/lipid binding motif is found at subdomains IB and IIIB, where the fatty acids are completely internalized within helical domain structures. Details of the complex and chemistry are discussed.

PS04.17.16 THE CRYSTAL STRUCTURE OF CARDIOTOXIN V FROM TAIWAN COBRA VENOM AT 2.19 Å RESOLUTION: ROLE OF WATER BINDING LOOP IN THE FORMATION OF MEMBRANE-BINDING SITE OF P-TYPE CARDIOTOXINS. Chwan-Deng Hsiao[1], Yuh-Ju Sun[1], Wen-guey Wu[2], Chien-Min Chiang[2], A-Yen Hsin[1],Crystallography Laboratory, Institute of Molecular Biology Academia Sinica, Taipei, Taiwan 11529[1]. Structural Biology Group, Department of Life Science, National Tsing Hua University, Hsinchu, Taiwan 30043[2]

The crystal structure of cardiotoxin V from Taiwan cobra venom (CTX A5) has been solved at pH 8.5. The refined model shows dimeric assembly and the global monomeric structure is found to be similar to that determined by NMR at pH 3.7. Nevertheless, local conformational differences are detected at two functionally important regions of loop I and II. The first difference between the NMR and X-ray structure of CTX A5 is detected near the tip of loop I and can be attributed to the different protonation state of His-4 at different pH. The second difference, detected at the tip of loop II, is due to the interaction of water with amino acid residues in the loop II region of the cardiotoxin containing Pro-31 (P-type CTX). This interaction forces the normally tapering flexible loop II into a more rigid Ω shape by bridging the main chain at 27 and 34 positions. Thus a continuous hydrophobic column capable of penetrating the membrane lipid bilayers is formed by the tips of three-finger toxin. These results provide a structural basis for the pH-dependent lipid binding activity of CTXs. In addition, a new membrane-spanning element other than helical and β-barrel structure is proposed by the hydrophobic loops of β-sheet polypeptides. Also discussed is a model of CTX cation channels to explain the cell lysis and depolarization activity.

PS04.17.17 NONNUCLEOSIDE RT INHIBITORS GIVE HIV-1 RT A CROOKED BACK. Yu Hsiou[1], Jianping Ding[1], Kalyan Das[1], Stephen H. Hughes[2], and Edward Arnold[1]* [1]Center for Advanced Biotechnology and Medicine (CABM) and Rutgers University Chemistry Department, 679 Hoes Lane, Piscataway, NJ 08854-5638, USA; [2]ABL-Basic Research Program, NCI-Frederick Cancer Research and Development Center, P.O. Box B, Frederick, MD 21702-1201, USA

The combined structural, biological, and genetic information on HIV-1 reverse transcriptase (RT) has enhanced our understanding of the mechanisms of polymerization and inhibition.