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.

Crystal structures of HIV-1 RT, either unliganded or complexed with different nonnucleoside RT inhibitors (NNRTI) or a double-stranded DNA, have been reported. There are significant differences between different HIV-1 RT structures; this serves to illustrate the high flexibility of this enzyme.

Comparison of various RT structures with or without bound ligand or substrate reveals that the p66 thumb subdomain can occupied at least three different positions that depend on whether RT interacts with or without substrate or various ligands. In contrast to the "thumb down" position found in the unliganded form, when HIV-1 RT binds a DNA substrate the p66 thumb is rotated open at the thumb's "knuckle", which is near residues Trp239 and Val317. This knuckle movement of the p66 thumb affects only the position of the p66 thumb, not other subdomains. The binding of an NNRTI, however, induces a hinge-like movement at the base of the p66 thumb subdomain, between the β6-β10-β9 and β12-β13-β14 ("primer grip") sheets. Associated with this hinge-like movement, the p66 thumb subdomain is further extended to a wide open position. The p66 connection subdomain, RNase H domain, and the subdomains in the p51 subunit are displaced by this hinge-like movement as well.

The polymerase active site is composed of structural elements from both protein and nucleic acid. NNRTI binding to HIV-1 RT leads to altered positions of both the p66 thumb and the primer grip, which consequently would alter the position of template-primer relative to both the polymerase and the RNase H active sites. Those conformational changes could account for the inhibition activity of NNRTIs, and explain the alteration of cleavage specificity of RNase H by NNRTI binding.

PS04.17.18 CYSTALLIZATION AND CRYSTALLOGRAPHIC STUDIES OF BAR-HEADED GOOSE DEOXYHAEMOGLOBIN. Ziqian Hua*, Yiling Fang, Xiaoxi Zhou, Qian Xu, Bao Kuang, Xincheng Wei, Guangying Lu, Xiaocheng Gu, College of Life Sciences,Peking University, Beijing 100871 China

Bar-headed goose (Anser indicus) live and hatch their young at the west China's Qinhai lake, but at the end of autumn they migrate to the plains of northwest India. Flocks has been observed flying over the Himalayan Mountains at altitudes of about 9000 m where ambient pO_2 only have about 50 mmHg which accounts to 20% of pO_2 at sea level (Swan, L. A. 1970, Nat. Hist., 79, 68).

The Hbs from bar-headed goose shows more high oxygen affinity compared to closely related lowland species of goose, such as grelag goose. There are only four amino acid differences between the major Hb types of these two species, only one of which appears likely to effect oxygen affinity, the α119 Pro matutes to Ala at $\alpha_1\beta_1$ interface(Oberthür, W., et al 1982, Hoppe-seyler's Z. Physiol. Chem.,363, 581)

We have determined the X-ray crystallographic structure of bar-headed goose haemogloblin in the Oxy form to a resolution of 0.2 nm, Now we have got the crystals of deoxyHb and done the preliminary crystallographic studies in order to elucidate high oxygen affinity mechanism. DeoxyHb of bar-headed goose was prepared with $Na_2S_2O_4$ as reducing agent. The single crystals suitable for X-ray analysis have been grown from PEG 6000 at pH 7.2, protein 20 mg/ml with the hanging drop vappor diffusion method. DeoxyHb crystallizes in a Pl space group with lattice constants a=7.09 nm, b=9.54 nm, c=5.87 nm, the asymmetric unit has two molecules, Vm=0.256 nm^3/Dal. The crystals diffract to about 0.23 nm resolution and 60% of X-ray diffraction data has been collected to 0.28 nm on X-200B Area Detector. A total of reflections is 30654, Rm=5.14% The determination of deoxyHb structure using the moleculer replacement method is in progress.

PS04.17.19 CRYSTALLIZATION AND PRELIMINARY X-RAY STUDIES OF PLASTOCYANIN FROM *SILENE* EXPRESSED IN *E. COLI*. T. Inoue*, M. Gotowda*, K. Hamada*, T. Takabe** & Y. Kai*.*Department of Applied Chemistry, Faculty of Engineering, Osaka University, Suita, Osaka 565, Japan, **Department of Chemistry, Faculty of Science and Technology, Meijo University, Tenpaku-ku, Nogoya 468, Japan.

Plastocyanins are type I copper proteins with a single polypeptide chain (10.5KDa). They have been found in a variety of higher plants and algae where they function in photosynthetic electron transport (Sykes, A. G., *et al.*, 1993). Plastocyanins are unique proteins among the blue copper proteins showing the two different electron transfer (ET) sites (Sykes, A. G., 1991, Qin, L. & Kostic, N. M., 1993). However, it still remains necessary to understand further the precise specificities of the two sites, the nature of the binding, and the intramolecular ET to and from in particular the remote site with different redox partners. In order to make clear the reletionships between their functions and structures, we have carried out the study on Crystallization and Preliminary X-ray Studies of Plastocyanin from *Silene* expressed in *E. Coli* (PCSIL). PCSIL has been crystallized in a form suitable for X-ray diffraction analysis by macroseeding method using ammonium sulfate as a precipitant in acetate buffer (P^H=5.5). These crystals belong to space group $P3_221$ with lattice parameters $a=b$=76.6 Å and c=65.5 Å, indicating an asymmetric unit containing two plastocyanin molecules. The crystals diffract up to at least 2.0 Å resolution. 44196 diffraction data were observed, from which 11515 were unique, in the resolution range 15.0-2.0 Å, with an *R*merge of 6.0%. Molecular replacement method was applied to solve the crystal structure with *AMoRe* in *CCP4*. Rigid-body refinement of the model and subsequent refinement using molecular dynamics were carried out with *XPLOR*, leading to a current R factor of 17.6%, for the diffraction intensities up to 2.5 Å resolution.

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PS04.17.20 PRELIMINARY CRYSTALLIZATION OF A CYCLIN-DEPENDENT KINASE INHIBITOR: P18. Shannon Jarchow and Hengming Ke Department of Biochemistry and Biophysics, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

Eukaryotic cell-cycle progression is regulated by a family of serine/threonine protein kinases, cyclin-dependent kinases (CDK's). CDK activity is regulated by subunit phosphorylation, activation via cyclin binding, and inhibition via binding of small regulatory proteins. Currently, these small inhibitor proteins can be classified into two families: the universal inhibitors of the p21/p27 family and the more specific inhibitors p16INK4 (p16)/p14INK4 (p14) family. A member of the p16/p14 family, p18 binds and inhibits CDK6 and CDK4, halting cell cycle progression.

Recombinant human p18 has been purified to homogeneity and diffraction quality crystals have been obtained by dialysis. Crystals have a typical size of 0.1 x 0.1 x 0.2 mm and can diffract to 2Å resolution. The space group has been determined to be P1 with unit cell dimensions of: a=60.3, b=40.2, c=28.4 Å, α=90.6°, ß=92.1°, γ=95.8°. Diffraction data have been collected on a RIGAKU R-AXIS image phosphate system. A data set of 16,785 measurements has been reduced to 11,595 independent reflection with an R-merge of 0.089. This data set is 70.5% complete to 2.06Å resolution. Heavy atom derivative preparation and structural analysis is in progress.