PS04.05.27 PROGRESS IN THE STRUCTURE DETERMINATION OF A HUMAN TOPOISOMERASE I - DNA COMPLEX. Matthew R. Redinbo[1], Lance Stewart[1], Ehmke Pohl[1], Xiayang Qiu[1], James J. Champoux[3], and Wim G. J. Hol[1,2], [1]Department of Biological Structure and [2]Howard Hughes Medical Institute, University of Washington, Seattle, WA 98195, [3]Department of Microbiology, University of Washington, Seattle, WA 98195

Progress in the structure determination of a complex of human topoisomerase I and double-stranded DNA will be described. Topoisomerases are a class of ubiquitous enzymes that control the level of DNA supercoiling in cells and are vital for transcription, translation and recombination. Topoisomerase I breaks a single strand of duplex DNA, forms a transient protein-DNA complex via a phosphotyrosine bond, and allows relaxation of superhelical strain about the intact DNA strand. Eukaryotic and prokaryotic topoisomerase I enzymes appear to be distinct and to have unique mechanistic characteristics. Human topoisomerase I is a 765 amino acid (91 kD) enzyme made up of four domains. Human topoisomerase I has been shown to be the sole target of the anti-cancer compound camptothecin and of camptothecin derivatives, and is thus of significant medical interest. Elevated levels of topoisomerase I have been identified in several human cancer cell types. We have obtained diffraction-quality crystals of a complex of a recombinantly expressed 70 kD portion of human topoisomerase I which includes the active site and double-stranded DNA. Data to 2.8 Å resolution has been collected at -170° C from native crystals and from crystals containing an iodinated DNA oligonucleotide. Structure determination is in progress using the multiple isomorphous replacement method.

PS04.05.28 CRYSTALLOGRAPHIC STUDIES OF THE ARCHAEAL INTRON ENCODED ENDONUCLEASE I-DmoI*I*. George H. Silva, Jacob Z. Dalgaard, Marlene Belfort, Patrick Van Roey, Wadsworth Center, New York State Dept. of Health, Albany, NY 12201-0509, USA

I-DmoI*I*, is a 22-kDa endonuclease encoded by an intron in the 23S rRNA gene of the hyperthermophilic archaeon *Desulfurococcus mobilis*. The endonuclease is thermostable with peak activity in the 65-75° C range *in vitro* and is capable of cleaving the intronless allele of the 23S rRNA gene. It recognizes an asymmetric target sequence and makes a staggered double-strand cut proximal to the intron insertion site generating 4-nucleotide 3'-OH extensions. A minimal binding site of 14-20 base pairs has been determined. I-DmoI*I*, requires a Mg^{++} co-factor for catalysis, but will readily bind DNA in its absence. I-DmoI*I*, contains the common LAGLI-DADG motif found in eukaryotic intron endonucleases. The motif consists of two twelve amino-acid sequences separated by about 100 non-essential residues. This motif is seen in proteins associated with DNA cleavage, RNA maturation and protein processing. Crystals of I-DmoI*I*, have been grown by the hanging drop method in PEG 3350, lithium sulfate and Tris.HCl pH 8.5. These crystals belong to the monoclinic space group C2 with unit cell dimensions a = 96.57 Å, b = 37.69 Å, c = 56.72 Å, β = 114.09° and V = 206,445 $Å^3$. Assuming two molecules per asymmetric unit yields a $V_M \approx 2.35$ $Å^3/D$, corresponding to about 48% solvent content. Native diffraction data have been measured to 2.3 Å resolution using flash cooling techniques. In addition, three possible derivative data sets have been obtained and are currently being used in phasing. Progress in the structure determination will be presented.

PS04.05.29 CRYSTAL STRUCTURAL ANALYSIS OF THREE SURFACE MUTANTS OF THE GENE V PROTEIN OF M13. ShaoYu Su, YiGui Gao, Howard Robinson, ++Hong Zhang, +Thomas C. Terwilliger, Andrew H.-J. Wang, Division of Biophysics & Dept. of Cell Structural Biology, University of Illinois at Urbana-Champaign, Urbana, IL 61801, +Division of Life Science & Structural Biology, Los Alamos National Laboratory, Los Alamos, NM 87545

The gene V protein (GVP) from the bacteriophage M13 is a single-stranded DNA binding protein which is a homo dimer of 87 amino acids. The high resolution structure of the M13 GVP has recently been determined by the multiwavelength anomalous diffraction method. In addition, the structure of the Y41H mutant has been determined at 1.7 Å resolution. On the basis of the three dimensional structures and the crystal packing interactions of both the wild-type and Y41H GVP, a model has been proposed to explain the cooperative nature of its binding to ssDNA. The model suggests possible involvements of many surface amino acids either in the binding to DNA, or the protein-protein interactions in the GVP-ssDNA complex. In this work, we present the crystal structures of three mutants involving surface amino acids, L32R, K69H and R82C. The hydrophobic L32 residue is converted into a basic arginine, whereas the basic K69 and R82 are converted into somewhat hydrophobic histidine and cysteine respectively. The diffraction data of these three mutants have been collected to 1.9, 2.0 and 2.0 Å, respectively. The structural refinements are in progress and the results will be reported.

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PS04.05.30 THE THREE DIMENSIONAL STRUCTURE OF AN ATP DEPENDENT DNA LIGASE FROM BACTERIOPHAGE T7. Hosahalli S. Subramanya, Aidan J. Doherty, Stephen R. Ashford and Dale B. Wigley, Laboratory of Molecular Biophysics, University of Oxford, South Parks Road, Oxford, OX1 3QU, UK

DNA ligase is a vital enzyme which is required for important cellular processes such as DNA replication, repair of damaged DNA and recombination. The enzyme mediates the formation of phosphodiester bonds between adjacent 3'-OH and 5'phosphate termini, thereby joining the nicks in the double stranded DNA. Ligases can be classified into two groups depending on their requirement for ATP or NAD^+ as the cofactor. All eukaryotic enzymes and virally encoded enzymes are ATP-dependent, whereas prokaryotic enzymes require NAD^+ for their activity. DNA ligase from bacteriophage T7 is a monomer with a molecular weight of 41 kDa. Here we report the structure of this enzyme at 2.6 Å resolution.

The protein was crystallized by vapour diffusion method using hanging drops. Crystals belonged to the space group $P2_12_12$ with unit cell dimensions a=65.8 Å, b=86.3 Å, c=78.3 Å. The structure was solved by MIR using mercury and selenomethionine derivatives. Non-isomorphism between the crystals was a major problem in structure determination. Crystals were found to be grouped around three major forms. The non-isomorphism between the crystal forms was sufficient to allow density averaging between them to improve the electron density maps.

The structure consists of two distinct domains, a larger N-terminal domain (residues 2-240) and a C-terminal domain (residues 241-349). The N-terminal domain is an α/β structure and comprises of three mainly antiparallel ß-sheets surrounded by six α-helices. The ATP-binding site is situated in this domain in a pocket beneath one of the β-sheets. The C-terminal domain con-