

accessible faces and the three four-stranded β -sheets. One therefore might characterize this fold as an inside-out α/β barrel. The present structure reflects the open conformation of the enzyme. We assume that large conformational changes upon catalysis are mediated mainly through two residues, a lysin and an arginine, which are located in the cleft between the two domains. These residues assure the open conformation of the native enzyme by the repulsive force between the positive charges of two amino groups. The binding of one of the two substrates probably results in neutralizing the repulsion between the two domains thereby allowing the movement of a catalytically active cysteine toward the cleft.

PS04.07.20 PROGRESS IN DETERMINING THE STRUCTURE OF FRAGMENT D FROM HUMAN FIBRINOGEN.

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Fragment D is a complex, large molecular weight (86,000 Da) fragment derived from vertebrate fibrinogen by limited proteolysis with plasmin or trypsin. Its three constituent polypeptide chains have long been supposed to form a coiled-coil at their amino-terminal ends and to be cemented by a ring-shaped triple set of disulfide bonds. The carboxyl-terminal portions of two of the chains make up two homologous globular domains. Recently we reported the crystallization of fragment D from human fibrinogen and some preliminary characterization (Everse *et al.* Prot. Sci., 4:101316, 1995). The space group is $P2_1$, unit cell dimensions $a = 107.7$, $b = 48.0$, $c = 167.6$, $\beta = 105.7$. In the interim, we have identified several isomorphous derivatives and have been able to calculate preliminary low resolution phases. The molecular envelope determined by solvent flattening of an initial Fourier map has revealed an silhouette in which the boundaries of the distal domains and coiled coil are reasonably delineated. It also reveals, in contrast to our initial finding, that there is only one molecule per asymmetric unit. The solvent flattened map itself contains several features which can be attributed to secondary structure, including portions of the coiled-coil. Efforts to improve the phasing are under way.

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PS04.07.21 CRYSTALLOGRAPHIC STUDIES OF THE HUMAN PENTRAXINS, SERUM AMYLOID P COMPONENT AND C-REACTIVE PROTEIN.

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The crystal structure of the two major human pentraxins have been determined to medium resolution. The pentraxins are a protein family which exhibit five fold symmetry of subunits and are capable of calcium dependent binding. The two major human pentraxins are Human Serum Amyloid P Component (SAP), which is found bound to amyloid deposits in amyloidosis and Alzheimer's disease, and C-reactive protein (CRP), which is believed to have a role during the immune response. SAP and CRP share 52% sequence identity. SAP exists in the plasma as a decamer, two pentameric rings interacting face to face, each subunit consisting of 204 amino acids, whereas CRP exists as a single pentameric ring made up of subunits consisting of 206 amino acids.

Two CRP crystal forms have been grown. Both are tetragonal. One has a unit cell of $a=b=275.81$ and $c=94.21$, and contains 3 pentamers per asymmetric unit and the other is of unit cell $a=b=190.31$ and $c=132.12$, space group $P4_32_12$, and contains two pentamers per asymmetric unit. A complete data set has been collected on this crystal form to a resolution of 3.0Å. Crystals have also been grown of decameric SAP. These are of space group $P2_1$ and unit cell $a=103.37$ $b=112.711$ $c=121.499$ and $\beta=91.87$ and contains 1 decamer per asymmetric unit. A complete data set has been collected on this crystal form to 2.5Å.

Both structures have been solved by molecular replacement using the pentameric structure of SAP (Emsley, J. *et al.* (1994) Nature vol. 367, p338-345) as a model.

PS04.07.22 CRYSTALS OF THE AUGMENTOR OF LIVER REGENERATION.

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Almost every school child knows that chameleons can regenerate a lost tail. This interesting phenomenon of regrowth of lost body parts common in lower animals is generally rare in mammals. The liver however, is a unique organ in that it is capable of regeneration.

A new cellular growth factor termed augmentor of liver regeneration (ALR) shown to have a proliferative effect on liver cells while at the same time producing an immunosuppressive effect on liver-resident natural killer cells and liver-resident mononuclear leukocytes has been crystallized. The crystals diffract to beyond 2 Å resolution and belong to space group $P2_12_12_1$, with $a=35.5$ Å, $b=108.1$ Å and $c=125.1$ Å. Based on 4 molecules per asymmetric unit, the Matthews coefficient is calculated to be 2.16 Å³/Da which corresponds to a solvent content of 43%.

Rat ALR has two related forms, "short" ALR which has 125 amino acid residues and "long" ALR, (ALR198), which contains 73 additional residues in the N-terminal segment of ALR. The long ALR is the result of an additional inframe ATG initiation site which is 5' to the initiation site used to code the 125 residue protein. The ALR cDNA sequence shows 50% homology with the sequence of the dual-function gene ERV1 (Essential for Respiration and Viability) of "baker's yeast", *Saccharomyces cerevisiae*. The ERV1 gene products are part of the mitochondrial respiratory chain, are essential for oxidative phosphorylation and vegetative growth and also play a critical role in cell growth and regulation. Deletion of this gene has been shown to cause a severe growth defect and irreversible cessation of cell division after 3-4 days. The ALR gene could represent the mammalian equivalent of the ERV1 gene.

Details of the structure analysis will be presented.

PS04.07.23 CRYSTAL STRUCTURE ANALYSIS OF BOVINE HEART CYTOCHROME C OXIDASE.

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Bovine cytochrome c oxidase is a large membrane protein complex with molecular size of 200KDa comprising 13 different subunits and six metal centers, heme a, heme a₃, Cu_A, Cu_B, Mg and Zn. As the terminal enzyme of biological oxidation, the enzyme catalyzes O₂ reduction to H₂O at an active site with the four redox active transition metals coupling to a proton pumping process across the mitochondrial inner membrane.

The structure of the enzyme has been determined at 2.8Å resolution. Electron transfer pathway has been elucidated by the structural study. A clear electron density map at 2.8Å resolution was obtained by m.i.r. method followed by a density modification method. Out of 3606 amino acid residues in an asymmetric unit composed of a dimer, structural models of 3560 residues as well as those of metal centers were successfully built.

The electron density map indicates a dinuclear copper center of Cu_A with an unexpected structure of [2Cu-2S_γ] similar to a [2Fe-2S] center. Zinc site is located at a nuclear encodes subunit on the matrix side

of the membrane. Magnesium site is situated between heme a_3 and Cu_A . The O_2 binding site contains heme a_3 iron and copper (Cu_B) atoms with a distance of 4.5 Å. There is no amino acid ligand bridging between the iron and the copper atoms in spite of a strong anti-ferromagnetic coupling between them. However, such a small bridging ligand between them as an oxygen atom is not excluded at present. The heme a and the heme a_3 are bridged by a polypeptide segment of His-Phe-His of which one histidine coordinates to the heme a iron and the other histidine to the heme a_3 iron.

The electron transfer path within the molecule has been established as follows: cytochrome $c \rightarrow Cu_A \rightarrow$ heme $a \rightarrow$ the O_2 binding site, which includes heme a_3 and Cu_B . Several hydrogen bonds providing electron transfer pathway were found between the Cu_A site and the hemes a . There exist several hydrogen bonds around the Cu_A , the heme a and the heme a_3 . The structure implicates that Cu_A -His²⁰⁴-CO of Arg⁴³⁸-NH of Arg⁴³⁹-a propionic group of the heme a is the primary electron transfer pathway between Cu_A and the heme a .

Hot Macromolecular Structures I

MS04.07a.01 THE STRUCTURE OF AN RNA PSEUDOKNOT THAT INHIBITS HIV-1 REVERSE TRANSCRIPTASE. Craig E. Kundrot, Cindy L. Barnes, and Susan E. Lietzke. Department of Chemistry and Biochemistry, University of Colorado, Boulder, CO 80309-0215.

An RNA pseudoknot inhibitor of HIV-1 reverse transcriptase was isolated by Tuerk, et al., using the *in vitro* selection technique SELEX. This 26 nucleotide RNA pseudoknot, PK26, specifically inhibits HIV reverse transcriptase with nanomolar affinity. PK26 and three bromo-uridine substituted derivatives were produced by chemical synthesis and crystallized from ammonium acetate. Two of the derivative crystals were suitable for data collection. Diffraction data were collected on cryocooled crystals stabilized with MPD. Native crystals diffracted to 2.9 Å and the derivatives to 3.0 Å. The crystals belong to space group $P4_3/122$ and have unit cell dimensions $a = b = 61.6$ Å, $c = 98.9$ Å. The bromine atoms in the two derivatives were located by Patterson methods and difference Fourier maps and used to produce an initial electron density map to 4 Å resolution (figure of merit = 0.49). The initial map clearly shows that the PK26 molecules coaxially stack in a head-to-head and tail-to-tail orientation. The structure of PK26 will be described and analyzed in terms of extensive chemical modification and nucleotide substitution data available for free PK26 and PK26-HIV RT. Since PK26 also conforms to the pseudoknot motif that promotes ribosome frame shifting, the structure will also be analyzed in terms of potential frame shifting mechanisms.

MS04.07a.02 CRYSTAL STRUCTURE OF HUMAN CYCLIN H, A CELL CYCLE REGULATORY PROTEIN. Kyeong Kyu Kim*, Holly M. Chamberlin†, David O. Morgan†, Sung-Hou Kim*. *Department of Chemistry and Earnest Orlando Lawrence Berkeley National Laboratory, University of California, Berkeley, CA 94720, USA, †Department of Physiology, University of California, San Francisco, CA 94143-0444, USA

The structure of human cyclin H was determined to 3 Å resolution by multiple isomorphous replacement. Cyclins are positive regulatory subunits that activate the catalytic subunits of cyclin-dependent kinases (CDKs) and trigger cell cycle events. In contrast to other cycling, cyclin H is the positive regulatory protein that activates the catalytic subunit, CDK7, which, in turn, activates most or all other CDKs. The crystals of cyclin H were grown in the presence of lithium and ammonium sulfate as precipitants, and belong to the space group $I4_122$ with unit cell dimen-

sions of $a = b = 84.0$ Å and $c = 374.7$ Å. Like cyclin A, cyclin H contains two α -helical domains that form a conserved cyclin fold. However, cyclin A and cyclin H show significant differences in the surface charge distribution and locations of the N- and C-terminal helices outside the cyclin fold. These differences may reflect the unique function of cyclin H.

MS04.07a.03 STRUCTURE OF Bcl-X_L, A DOMINANT INHIBITOR OF PROGRAMMED CELL DEATH. Steven W. Muchmore¹, Micheal Sattler², Heng Liang², Robert P Meadows², John E. Harlan², David Nettlesheim², Brian Chang³, Craig B. Thompson³, Sui-Lam Wong⁴, Shi-Chung Ng⁴, Stephen W. Fesik², ¹Protein Crystallography, ²NMR Research, ³Aging and Degenerative Disease Research, Pharmaceutical Products Research Division, Abbott Laboratories, Abbott Park, IL 60064, USA, ⁴Howard Hughes Medical Institute and Departments of Medicine, Molecular Genetics, and Cell Biology, University of Chicago, Chicago, IL 60637, USA.

The Bcl-2 family of proteins modulates programmed cell death (apoptosis) by an unknown mechanism. The structure of Bcl-X_L was solved by a combination of x-ray crystallographic and NMR spectroscopic techniques. The structure consists of a total of 7 α -helices, two of which form a central apolar pair. The remaining helices are amphipathic and flank the central pair. A loop of approximately 60 residues connecting the first two helices was found to be both flexible and dispersible for anti-apoptotic activity. Three functionally important homology domains (BH1, BH2, BH3) are located on the same face of the molecule and form a hydrophobic cleft which may represent the interaction site for proteins which promote apoptosis. Sequence alignments of other Bcl-2 family members suggests these proteins should exhibit the same overall fold. The Bcl-X_L structure is reminiscent of membrane translocation domains of bacterial toxins such as diphtheria toxin or colicin. This similarity suggests that the modulation of apoptosis by the Bcl-2, family of proteins may involve a membrane translocation event.

MS04.07a.04 CRYSTAL STRUCTURE OF THE IMMUNODOMINANT CHAPERONIN-10 OF MYCOBACTERIUM LEPRAE. Shekhar C. Manda^{a,*}, Vijay Mehra^b, Barry R. Bloom^{b,c} and Wim G. J. Hol^d. ^aInstitute of Microbial Technology, Chandigarh 160 036, India; ^bDepartment of Microbiology and Immunology, and ^cHoward Hughes Medical Institute, Albert Einstein College of Medicine, Bronx, New York, USA; and ^dBiomolecular Structure Centre and the Howard Hughes Medical Institute, University of Washington, Seattle, WA 98195 USA.

Mycobacteria are amongst the most important human pathogens. In an effort to understand the immune response to mycobacterial infections, several antigens of these microbes have been identified. A 10kD antigen has been found to be the most immunogenic protein antigen of *M. leprae*. Interestingly it shares ~44% sequence identity with the well known chaperonin GroES of *E. coli* (1). We present the three dimensional structure of this protein, with possible hypothesis on the role of cpn-10 in the chaperonin mediated protein folding process.

The heptameric molecule has a dome like structure, with approximate dimensions of 80x80x35 angstroms (2). The overall architecture is strikingly similar to the pantheon in Rome. Residues important for its interaction with the larger chaperonin partner, cpn-60 (GroEL homologues) are sequestered on the lower surface of the dome. The interior of the dome is intensely hydrophilic. Residues lining the interior surface of the dome are conserved evolutionarily suggesting that GroES may take an active part in chaperonin mediated protein folding process.

- (1) Mehra V et al., J. Exp. Med. (1992) v.175, 275-284.
- (2) Manda S C et al., Science (1995) v.271, 203-207.