PS04.07.16 CRYSTAL STRUCTURE OF CYANOBACTERIAL PHOTOLYASE (DNA PHOTOREACTIVATING ENZYME) FROM *Anacystis nidulans*. K. Miki[1], T. Tamada[1], K. Kitadokoro[1], Y. Higuchi[1], A.Yasui[2], P. E. de Ruiter[3], A. P. M. Eker[3], [1]Department of Chemistry, Graduate School of Science, Kyoto University, Sakyo-ku, Kyoto 606-01, Japan, [2]Institute of Development, Aging and Cancer, Tohoku University, 4-1 Seiryomachi, Aoba-ku, Sendai 980, Japan, [3]Department of Cell Biology and Genetics, Medical Genetics Centre, Erasmus University Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands

The crystal structure of photolyase (DNA photoreactivating enzyme) from the cyanobacterium, *Anacystis nidulans*, has been solved by the multiple isomorphous replacement. The refinement of the structure is still in progress and the present R value is 0.24 at 2.5 Å resolution.

Photolyase has activities of DNA damage recognition and binding, photon absorption, energy transfer from chromophore to DNA, resulting in photoreversal of UV-induced pyrimidine dimer to monomer. Photolyases are single chain proteins of 50 to 70 kDa molecular weight, containing two different chromophores in equimolar amount. Deduced FAD was found to be an essential chromophore for the lights-dependent repair process and the second cofactor besides FAD is 8-hydroxy-5deazaflavin (8-HDF) in *A. nidulans* photolyase with absorption maxima around 440 nm. On the other hand, 5,10-methenyltetrahydrofolic acid (MTHF) is the second cofactor with absorption maxima around 380 nm in *E. coli* photolyase whose structure was recently determined (Park *et al.*, Science, 268, 1866, 1995).

Photolyase from *A. nidulans* crystallized in the both tetragonal and trigonal systems (Miki *et al.*, J. Mol. Biol., 233, 167, 1993; Tamada *et al.*, J. Struct. Biol., 115, 37, 1995). The present structure analysis by the use of the tetragonal crystals showed that the whole folding pattern is essentially similar to that of *E. Coli* photolyase but that the binding mode of the second chromophore is different.

PS04.07.17 STRUCTURE OF MACROPHAGE MIGRATION INHIBITORY FACTOR FROM HUMAN LYMPHOCYTES. A.Nakagawa*, H.Sugimoto*, M.Suzuki§, I.Tanaka* and J.Nishihira† *Division of Biological Scieces, Graduate School of Science, Hokkaido Univ., Sapporo 060, Japan, §Photon Factory, National Laboratory for High Energy Physics, Tsukuba 305, Japan, †Central Research Institute, School of Medicine, Hokkaido University, Sapporo 060, Japan

The three-dimensional sructure of macrophage migration inhibitory factor (MIF) from human lymphocytes was solved at 2.1Å resolution by the molecular replacement. MIF was suggested to concentrate macrophages at the infection site and make them function in antigen processing and phagocytosis. MIF is also a potent activator of macrophages and is likely to be critical in cell-mediated immune host defenses. However, actual function and mechanism of macrophage migration inhibitaion of MIF is still unknown. We have reported the structure of MIF from rat liver and unexpected simularites between MIF and two isomerases (Suzuki *et al.*, *Nature Struct. Biol., in press*, 1996). Crystal of rat-MIF contains one monomer, which consists of two $\beta\alpha\beta$ motifes aligned quasi two-fold symmetry, in an asymmetric unit. However, the crystal structure shows that the protein forms a trimer by inter-subunits β-sheets. The MIF trimer, which we call trimeric β-cage, is formed by three five-stranded (one β-strand comes from a next monomer) β-sheets, which surrounded by six α-helices. The electron density of MIF from rat liver does not show clear electron density at the carboxyl terminal, and the model of rat-MIF doesn't have C-terminal eleven residues.

The space group of crystal of the MIF from human lymphocytes is $P2_12_12_1$(a=68.4,b=68.8,c=86.8Å). This crystal contains one MIF trimer in an asymmetric unit, and we used rat-MIF trimer structure as a starting model for molecular replacement. Rotation and translation searches were calculated by X-PLOR (Brünger, *X-PLOR version 3.1 Manual*, 1993). After the first cycle of simulated anneaing refinent, the (2Fo-Fc)-map showed a clear electron density at the C-terminal region. The structure of human-MIF has additional two β-strands, those make β-sheet structure with neighbouring MIF monomer. Thus, the structure of human-MIF has seven-stranded β-sheets, which consist from three monomers. The crystallographic R-factor of the current structure is 24% (R_{Free}=29%) at 2.1Å, and refinement is in progress.

PS04.07.18 X-RAY STRUCTURE DETERMINATION OF HUMAN GRO/MGSA PROTEIN. Hirofumi Ohishi*, Mitsuaki Sugawara#, Hironobu Nakayama#, and Ken-ichi Tomita*. Osaka University of Pharmaceutical Sciences, Matsubara, Osaka 580; #Faculty of Pharmaceutical Sciences,Osaka University, Suita, Osaka 565, Japan.

The human GRO (growth related protein)/ MGSA (melanoma growth stimulatory activity) protein with 73 amino acid (1) is a member of a super- family of chemotactic cytokineslike interleukin-8, platelet factor-4 (PF-4) monocyte chemoattractant factor and macrophage inflammatory protein-2. The human GRO gene was chemically synthesized and the secreted recombinant GRO protein expressed in *E.coli* was purified by chromatography, and used for crystallization by vapor diffusion. After trials with several different precipitants, the crystals grew as regular bipyramids with cubic cell dimensions: a=b=c=120.44 Å and space group I432, z=48 (dimeric GRO molecule per asymmetric unit). Intensity data up to 2 Å resolution were collected by RIGAKU R-AXIS imaging plate detector. The structure was solved by the molecular replacement method using the atomic coordinates for the dimer of PF-4(2) (from Protein Data Bank). and was then refined with 2.2 Å X-ray data, using CCP4, O and X- PLOR programs. The current overall basic structure found in GRO crystals is similar to the dimeric solution model by NMR spectroscopy(3), but the less restrained regions, the NH_2- and COOH-terminal regions are different from that of the PF-4 crystal structure and also of GRO NMR structure.

References:
1) A. Anisowicz et al., Proc. Natl. Acad. Sci. USA., 84, 718 (1987)
2) R. St. Charles et al., J. Biol. Chem., 264, 2092 (1989)
3) K-S. Kim et al., J. Biol. Chem., 269, 32909 (1994)

PS04.07.19 CRYSTAL STRUCTURE OF ENOLPYRUVYL-TRANSFERASE, THE TARGET OF THE ANTIBIOTIC FOSFOMYCIN. E. Schönbrunn[1], A. Perrakis[2], S. Eschenburg[2], F. Krekel[3], N. Amrhein[3], E. Mandelkow[1], and S. Sack[1], [1]Max-Planck Unit, c/o DESY, D-22607 Hamburg Germany, [2]EMBL, c/o DESY, D22607 Hamburg Germany, [3]ETH Zurich, Inst. Plant Sciences, CH-8092 Zurich, Switzerland

The crystal structure of Enolpyruvyltransferase (EPT) reveals an unusual fold and suggests large conformational changes upon catalysis. EPT catalyses the first committed step in the biosynthesis of the bacterial cell wall and is of potential pharmaceutical interest because it is irreversibly inhibited by the broad spectrum antibiotic fosfomycin. The crystal structure of native EPT consists of two distinct globular domains which are connected to each other by a double-stranded hinge. The most striking feature of the structure is the six-fold repetition of one folding unit or subdomain. Although these subdomains are remarkably similar in secondary structure elements and fold, the only repetitive element in the amino acid sequence is a small motif LXXXG(A) which is part of a loop connecting a helix with a beta strand. This motif is responsible for the attachment of the folding units to each other. The core of both domains consists of three α-helices which are surrounded by three helices with solvent

accessible faces and the three four-stranded ß-sheets. One therefore might characterize this fold as an inside-out α/β barrell. 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 cystein toward the cleft.

PS04.07.20 PROGRESS IN DETERMINING THE STRUCTURE OF FRAGMENT D FROM HUMAN FIBRINOGEN. G. Spraggon, S. J. Everse, R. F. Doolittle, Center for Molecular Genetics, Univ. Calif. San Diego, La Jolla, CA, USA 92093-0634

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.

Supported by N.I.H. Grant HL-26873. SJE was supported by a Fellowship from the American Heart Association, California Affiliate.

PS04.07.21 CRYSTALLOGRAPHIC STUDIES OF THE HUMAN PENTRAXINS, SERUM AMYLOID P COMPONENT AND C-REACTIVE PROTEIN. D. Thompson, I. J. Tickle, T. L. Blundell, M. B. Pepys[1] & S. P. Wood[2]. Department of Crystallography, Birkbeck College, Malet Street, London, [1]Immunological Medicine unit, Hammersmith Hospital, London, [2]Department of Biochemistry, University of Southampton, Southampton

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 spacegroup $P2_1$ and unit cell a=103.37 b=112.711 c=121.499 and β=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. C.-K. Wu[1,2], J.P. Rose[1], A. Francavilla[3], J.G. Prelich[3], A. Iacobellis[3], M. Hagiya[4], A. Rao[3], T. Starzl[3] and B.C. Wang[1], [1]Dept. of Biochemistery and Molecular Biology, Univ. of Georgia, Athens GA 30602, USA, [2]Dept. of Crystallography, Univ. of Pittsburgh, Pittsburgh, PA 15260, USA, [3]Pittsburgh Transplant Inst., Univ. of Pittsburgh School of Medicine, Pittsburgh, PA 15260, USA and [4]Pharmaceuticals Res. Ctr., Toyobo Co. Ltd., Ohtsu, Shiga 520-02, Japan

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 an 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=35.5Å, b=108.1Å and c=125.1Å. 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 phosphorlyation 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. H. Yamaguchi, T. Tsukihara, H. Aoyama, E. Yamashita, T. Tomizaki, K. Shinzawa-Itoh*, R. Nakashima,* R. Yaono* and S. Yoshikawa*. Institute for Protein Research, Osaka University, Suita, Osaka565, Japan; and *Faculty of Science, Himeji Institute of Technology, Kamigohori, Ako-gun, Hyogo678-12, Japan

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 a_3, Cu_A, Cu_B, Mg and Zn. As the terminal enzyme of biological oxidation, the enzyme catalyzes O_2 reduction to H_2O 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 dimmer, 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-2Sγ] similar to a [2Fe-2S] center. Zinc site is located at a nuclear encodes subunit on the matrix side