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:
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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