PS04.02.41 CRYSTAL STRUCTURE OF CARBOXYPEPTIDASE G_2 AND COMPARISON WITH OTHER ZINC-CONTAINING EXOPEPTIDASES. Siân Rowsell[1], Richard A. Pauptit[2], Alec D. Tucker[2], Peter Brick[1], Lesley F. Lloyd[1], Roger G. Melton[3], David M. Blow[1]. [1]Blackett Laboratory, Imperial College, London SW7 2BZ, UK, [2]Protein Structure Laboratory, Zeneca Pharmaceuticals, Macclesfield, Cheshire, SK10 4TG, UK, [3]Division of Biotechnology, PHLS Centre for Applied Microbiology and Research, Porton Down, Salisbury, SP4 0JG, UK

Enzymes of the carboxypeptidase G class hydrolyse the C-terminal glutamate moiety from folic acid and analogues such as methotrexate. Carboxypeptidase G_2 (CPG_2) is a zinc metalloenzyme produced by *Pseudomonas* sp. strain RS-16. There is no amino-acid sequence homology with other carboxypeptidases for which structural information is available.

Cancer therapies often rely on the plasma depletion of reduced folates which are essential cofactors in purine and pyrimidine biosynthesis. When coupled to antibodies which target the tumour cells, CPG_2 is potentially useful in activating prodrugs at the tumour site. CPG_2 may also be used to remove excess methotrexate from circulation in patients having prolonged treatments with this relatively toxic agent.

The crystal structure of CPG_2 has been determined to 3.0 Å resolution by the technique of multiple isomorphous replacement. The current crystallographic *R*-factor is 21.9% for all reflections between 10.0 and 3.0 Å resolution.

The CPG_2 molecule is a dimer composed of subunits of molecular mass 41,800 Da. The subunit consists of a catalytic domain, which contains a cocatalytic zinc site, and a second domain, which forms a dimer interface through hydrophobic interactions as well as through hydrogen bonding between two symmetry-related β-strands. The catalytic domain has close structural similarity to several zinc-containing exopeptidases. The topology of the second domain is similar to several RNA-binding domains. A structural comparison between CPG_2 and other zinc-containing exopeptidases suggests a remote divergent evolutionary relationship between these enzymes.

PS04.02.42 INSERTION OF A BULKY RESIDUE ADJACENT TO THE PROTON SHUTTLE GROUP IN CAII SIGNIFICANTLY DECREASES THE EFFICIENCY OF PROTON TRANSFER. Laura Scolnick*, Jane E. Jackman#, Kenneth M. Merz+, Jr., David W. Christianson*, Carol A. Fierke# *Department of Chemistry, University of Pennsylvania, Philadelphia, PA 19104-6323. #Department of Biochemistry, Duke University Medical Center, Box 371, Durham, North Carolina 27710, +Chemistry Department, Pennsylvania State University, University Park, PA 16802

Human carbonic anhydrase II (CAII) is a zinc metalloenzyme that catalyzes the reversible hydration of CO_2 to HCO_3^- and a proton in a two-step mechanim. First, zinc-bound hydroxyl attacks the carbonyl carbon of CO_2 to form a zinc-bound bicarbonate that is subsequently replaced by water to release product bicarbonate. The second step, which regenerates the active site zinc-hydroxide species, involves the transfer of a proton from zinc-bound water to a solvent buffer molecule,this is the rate-limiting step for CO_2 hydration at high substrate concentrations. Kinetic data and high resolution x-ray crystallographic structures of *E. coli* expressed CAII variants A65F, A65H, A65L, A65T, A65S, and A65G reveal that larger amino acids substituted at position 65 protrude into the active site and block proton transfer between the active site zinc bound water and histidine 64. In addition, evolutionary drift of position 65 among the CA isozymes shows that position 65 has evolved to be a small amino acid.

PS04.02.43 ANALYSIS OF THE SUBSTRATE SPECIFICITY AND THE ENZYMATIC ACTIVITY OF AN EXTRADIOL TYPE DIOXYGENASE. T.Senda, H.Narita, K.Taguchi, K.Sugimoto, E.Masai, M.Fukuda and Y.Mitsui, Department of BioEngineering, Nagaoka, University of Technology, Nagaoka, Niigata 940-21, JAPAN.

The substrate specificity and the enzymatic activity of the "BphC" enzyme have been analyzed applying crystallographic and biochemical techniques for several mutant proteins. The BphC enzyme is a member of the extradiol type dioxygenases and known as a key enzyme in the PCB degrading pathway of microorganisms. We have already solved the three-dimensional structure of the free form of the BphC enzyme at 1.8 Å resolution and the substrate-bound forms at 2.6 Å resolution[1]). The active site of the enzyme is situated inside the ß-barrel-like structure in the C-terminal domain. The amino acid residues in contact with the catechol ring moiety of the substrate, which is cleaved by the enzyme, are well conserved among the related enzymes indicating the almost identical catalytic mechanism within the extradiol type dioxygenase family. On the other hand, amino acids around the benzene ring moiety of the substrate are less conserved. Thus these residues seem to determine the substrate specificity of the enzyme. To analyze the role of these amino acid residues in detail, we have prepared the mutant proteins and determined the crystal structures of these mutants as well as the kinetic parameters, K_m and k_{cat}. These structural and biochemical analyses have revealed clearly that 1) Ile174, Thr280 and Phe201 affect the substrate specificity, 2) the amino acid residues bound to the Fe ion in the active center are essential to the enzymatic activity, and 3) His194, His240 and Tyr249, which are located close to the catechol ring moiety of the substrate, are also essential to the activity of the enzyme.

1) Sugiyama *et al.* (1995) *Proc Japan Acad.* **71B**, 33-35.; Senda *et al* (1996) *J.Mol. Biol.* 255, 735-752

PS04.02.44 TOWARD THE ALLOSTERIC MECHANISM OF FRUCTOSE-1,6-BISPHOSPHATASE: X-RAY STRUCTURES AND KINETICS OF MUTANT ENZYMES. Boguslaw Stec, Guqiang Lu, Reimar Abraham, Eugene Giroux, Mark K. Williams, Evan R. Kantrowitz, Department of Chemistry, Merkert Chemistry Center, Boston College, Chestnut Hill, MA 02167.

Fructose-1,6-bisphosphatase (Fru-1,6-P_2ase, E.C. 3.1.3.11) is a tetrameric enzyme playing an important role in a metabolic control of gluconeogenesis. Each identical subunit has 337 amino acid residues and a molecular weight of about 36,500. The physiological regulators AMP and Fru-1,6-P_2 negatively modulate enzyme activity and, in reciprocal fashion, positively affect the activity of phosphofructokinase, a control point in glycolysis.

Fru-1,6-P_2ase from pig kidney was cloned and expressed in *E. coli.* A series of mutant enzymes were created in order to study the allosteric and catalytic mechanism of its regulation. Mutant proteins were isolated, kinetically characterized, and crystallized in the T and R-allosteric states. Protein in the T state usually crystallized in P21212 space group with cell dimensions a= 61Å, b=166Å, c=79Å, and the R-state in P3221 space group with cell dimensions a=b=131Å, c=66Å in a hexagonal lattice.

The mutants: Arg243→Ala, Lys42→Ala and Arg22→Ala were prepared by site-specific mutagenesis and kinetically characterized. The mutant enzymes were isolated and crystallized. Data were collected on the Hamlin Are Detector to the resolution of 2Å, 2.2Å and 2.7Å, respectively. Structures were refined by X-plor and characterized.

Preliminary interpretation of the correlation between the kinetic results and the X-ray structures will be presented. These data suggest that the Arg243 is important for the proper inhibition by Fru-2,6-P_2, Arg22 is important for the T-state stabilization and Lys42 for the transmission of the allosteric signal from the AMP binding site to the catalytic site which are more than 20Å apart.