Enzymes of the carboxypeptidase G class hydrolyse the C-terminal glutamate moiety from acidic and related proteases such as methotrexate. Carboxypeptidase G2 (CPG2) 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, CPG2 is potentially useful in activating prodrugs at the tumour site. CPG2 may also be used to remove excess methotrexate from circulation in patients having prolonged treatments with this relatively toxic agent.

The crystal structure of CPG2 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 CPG2 molecule is a dimer composed of subunits of molecular mass 41,800 Da. The subunit consists of a catalytic domain, which contains a catalytic 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 CPG2 and other zinc-containing exopeptidases suggests a remote divergent evolutionary relationship between these enzymes.

A series of mutant enzymes were created in order to study the substrate specificity of the enzyme. The 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, *Km* and *Vmax*. These structural and biochemical analyses have revealed clearly that 1) Ile74, 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 catalytic ring moiety of the substrate, are also essential to the activity of the enzyme.

Fructose-1,6-bisphosphatase (Fru-1,6-P2ase, E.C. 3.1.3.11) is a tetramer 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-P2 negatively modulate enzyme activity and, in reciprocal fashion, positively affect the activity of phosphofructokinase, a control point in glycolysis.

Fructate-1,6-bisphosphatase from pig kidney was cloned and expressed in *E. coli*. A set 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 P2221 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-P2, 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.