P.04.14.17

Acta Cryst. (2005). A**61**, C241

Structure of Cytokinin-specific Binding Protein in Complex with Plant Hormone

Oliwia Pasternak[a], Grzegorz D. Bujacz[a,b], Yasuyuki Fujimoto[c], Yuichi Hashimoto[4], Michal M. Sikorski[a], Mariusz Jaskolski[a,e], *[a]Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland. [b]Institute of Technical Biochemistry, Technical University of Lodz, Poland. [c]Faculty of Pharmaceutical Sciences, Teikyo University, Japan. [d]Institute of Molecular and Cellular Biosciences, University of Tokyo, Japan. [e]Department of Crystallography, A. Mickiewicz University, Poznan, Poland.* E-mail: yenefer@rose.man.poznan.pl

The high-resolution (1.2 Å) crystal structure a cytokinin-specific binding protein from mung bean (VrCSBP) complexed with zeatin reveals that the protein, structurally resembles plant pathogenesis related proteins of class 10 (PR-10), despite a low sequence conservation (below 20%). The four VrCSBP molecules present in the asymmetric unit assemble into two dimers. Between the concave face of the molecular β-sheet and the C-terminal helix, a binding pocket is formed where the zeatin molecules are located. Surprisingly, in three (out of the four) binding pockets two zeatin molecules are found, with excellent definition in the electron density maps. In one of the binding sites (observed also in the forth, single-site, VrCSBP molecule), the ligand molecules, located deep in the cavity, have identical conformation and hydrogen-bonding pattern. In the second binding site, at the entrance to the internal cavity, the ligand molecules show variable, but clearly defined, binding modes.

Keywords: protein-ligand complexes, plant hormones, high resolution X-ray structures macromolecules

P.04.14.18

Acta Cryst. (2005). A**61**, C241

Modular Assembly of the Cellulosome Revealed by X-ray Crystallography

Maria J. Romão[a], Ana L. Carvalho[a], J. Prates[b], Gilbert[c], G. Davies[d], C. Fontes[b], *[a]REQUIMTE/CQFB, Dept. Química, FCT-UNL, 2829-516 Caparica, Portugal. [b]CIISA-FMV-UTL, 1300-477 Lisboa. [c]Univ. Newcastle, Newcastle/Tyne NE1 7RU, UK. [d]Struct. Biol. Lab, Univ. York, Heslington, York YO10 5YW*. E-mail: mromao@dq.fct.unl.pt

Clostridium thermocellum is an anaerobic bacterium isolated from hot springs which converts hemicellulose into ethanol. These microorganisms express multienzyme complexes dedicated to the degradation of the plant cell wall. These complexes (cellulosomes) are composed of modules assembled by an integrating protein (scaffoldin), composed of several type I cohesins, which bind type I dockerins. A type II dockerin of the scaffoldin binds to a type II cohesin and anchors the whole complex to the cell. Other modules named Carbohydrate Binding Modules (CBM), are responsible for adherence to the substrate.

The crystal structure of type I cohesin-dockerin complex was solved to 2.2 Å and revealed for the first time how protein-protein recognition is achieved in the complex [1]. The 2.5 Å crystal structure of the type II cohesion, solved by MIR/MAD will be described. Subtle differences between type I and type II cohesins give insight into the structural determinants of cohesin-dockerin specificity. We will also report the 1.98 Å structure (MAD-SeMet) of the family 11 CBM belonging to a cellulosomal enzyme. The structure of the CBM11 reveals a concave side that forms a potential carbohydrate binding cleft [2].

[1] Carvalho A.L., et al., *Proc. Natl. Acad. Sci. USA*, 2003, **100**, 13809-14. [2] Carvalho A.L., et al., *J. Biol: Chem.*, 2004, **279**, 34785-93.

Keywords: cellulosome, cohesin, carbohydrate binding module

P.04.15.1

Acta Cryst. (2005). A**61**, C241

Structural Studies of Thioredoxins and Associated Inhibitor Based Complexes

Gareth Hall, Manish Shah, Charlie Laughton, Andrew Westwell, Jonas Emsley, *Centre for Biomolecular Science, University of Nottingham, Nottingham, UK*. E-mail: paxgh@nottingham.ac.uk

The thioredoxin redox system is ubiquitous in all living cells and is used as a sophisticated mechanism for maintaining an intracellular reduced state. The redox proteins are also known to be important in a multitude of biological functions, including controlling cell cycle regulation, and studies in various human malignancies and cell lines in *vitro* have shown an up regulation of thioredoxin, demonstrating a definite link between thioredoxin and cancer [1], [2].

There are currently two novel heteroaromatic quinol inhibitors under development at the Cancer Research Laboratories of the University of Nottingham. These inhibitors are thought to have a novel mode of action leading to an irreversible binding of the inhibitor to the active site, thus irreparably inactivating the protein.

The research group has obtained the crystal structures of *Tuberculosis Bacterium* and human thioredoxins. By studying the crystal structure of thioredoxin-inhibitor complex it will be possible to apply structure-activity relationships and thus enable the research group to not only understand how these quinols block the activity of thioredoxin, but also to develop these drugs with the intention of improving their affinity for the binding site.

[1] Arrigo A.P., *Free Radical Biol. and Med.*, 1999, **27 (9/10)** 936. [2] Soini Y., et al., *Clinical Can. Res*., **7**, 1750.

Keywords: thioredoxin, cancer, heteroaromatic quinols

P.04.15.2

Acta Cryst. (2005). A**61**, C241

A Dramatic Side Chain Movement in Adrenaline-Synthesising PNMT: Implications for Drug Design

Christine L. Gee[a], Joel D. Tyndall[b], Gary L. Grunewald[c], Michael J. McLeish[d], Jennifer L. Martin[a], *[a]Institute for Molecular Bioscience and Special Reseach Centre for Functional and Applied Genomics, The University of Queensland, Australia. [b]National School of Pharmacy, University of Otago, New Zealand. [c]Department of Medicinal Chemistry, The University of Kansas USA. [d]College of Pharmacy, University of Michigan, USA*. E-mail: c.gee@imb.uq.edu.au

Phenylethanolamine N-methyltransferase (PNMT) catalyses the methylation of noradrenaline to form adrenaline using S-adenosyl-L-methionine as the methyl donor. Adrenaline is produced in the adrenal medulla (hormone), and in selected neurons in the CNS (neurotransmitter). The role of adrenaline in the CNS is poorly understood, though it has been implicated in blood pressure control and Alzheimer's disease.

Classic inhibitors of PNMT also act on the α2-adrenoreceptor, or are unable to cross the blood brain barrier. Therefore we are using the crystal structure of PNMT to design potent selective CNS-active PNMT inhibitors. The structure of PNMT with 7-SO_2NH_2-THIQ[1] revealed room in the binding pocket for bulkier 7 substituents so these were designed and tested for PNMT inhibition. Some inhibited with high potency despite predicted steric clashes. A co-crystal structure revealed a dramatic conformational change in a lysine residue to accommodate the substituent, indicating that drug design strategies must address large conformational changes at active sites.

[1] Martin J.L., Begun J., McLeish M.J., Caine J.M., Grunewald G.L., *Structure*, 2001, **9**, 1.

Keywords: enzyme inhibitor drug design, structure-based drug design, protein flexibility

P.04.15.3

Acta Cryst. (2005). A**61**, C241-C242

Structural Studies of Glutathione S-transferase Inhibitors – A Promising Target for Anti-cancer Drug Design

Lorien Parker[a], Julian Adams[a], Mario Lo Bello[b], Michael Parker[a], *[a]St. Vincents Institute, Melbourne. [b]Department of Biology University of Rome, Rome, Italy*. E-mail: lparker@svi.edu.au

Glutathione S-Transferases (GSTs), phase II detoxification enzymes, primarily function to detoxify unwanted toxic compounds in the cell [1]. They are, however, overexpressed in many cancers and shown to be deleterious to cancer chemotherapy's success by reacting with certain anti-cancer drugs. GSTs, therefore, have been identified

as an attractive target for inhibitor drug design to increase the efficacy of treatment [2].

Drug resistance remains a limiting factor in cancer chemotherapy and thus understanding the mechanisms of this effect represents an essential step in improving cancer treatment. There are many reports correlating over-expression of GST and reduced sensitivity to chemotherapy in lung, liver, breast, ovarian, and other forms of cancer[1]. GSTs are hypothesized to catalyse conjugation of GSH to anticancer drugs forming inactive conjugates, therefore, decreasing efficacy in treatment. The precise mechanisms responsible for the development of resistance to these commonly used anti-cancer agents is currently unknown. Gaining insight, through structural studies by X-ray crystallography, of this enzyme complexed to these compounds, will aid in the design of effective, and specific, inhibitors.

One of the major aims of this work is to determine the 3D structures of these complexes and subsequently pursue structure-based drug design of human GST pi class enzyme (hGSTP1-1) with the hope of discovering potent specific inhibitors. I have collected over 25 data sets of GST complexed to a range of compounds, several of which have been solved and the structures completed. The structure of the hGSTP1-1 in complex with these compounds will identify critical residues which will aid drug design of novel, therapeutic, GST inhibitors.

[1] Sheehan D., et al., *Biochem. J*., 2001, **360**, 1. [2] Farmer G., *Nature Rev. Drug. Discov.*, 2004, **3**, 547.

Keywords: anticancer drugs, inhibitor design, protein crystallography drug design

P.04.15.4

Acta Cryst. (2005). A**61**, C242

X-ray Crystallograpy of the Antiepileptic Drug Zonisamide with CA II

Angela Casini[a], Giuseppina De Simone[b], Carlo Pedone[b], Jochen Antel[c], Andrea Scozzafava[a], Claudiu T. Supuran[a], [a]*Dipartimento di Chimica, Università degli Studi di Firenze, Florence, Italy.* [b]*Dipartimento di Chimica Biologica-Sezione Biostrutture and Istituto di Biostrutture e Bioimmagini-CNR, University of Naples "Federico II", Naples, Italy.* [c]*Solvay Pharmaceuticals, Hannover, Germany.* E-mail: angela.casini@tiscali.it

Zonisamide, **ZNS**, is a widely used antiepileptic drug [1] whose mechanism of action is still not fully clarified. Recently a patent[2]claimed that ZNS is also effective for reducing weight in obese subjects and for treating eating disorders.

In a previous study we showed by means of solution and X-ray studies, that another sulfonamidic antiepileptic drug, Topiramate, is a strong inhibitor of physiologically relevant human carbonic anhydrase (hCA) [3].Thus we decided to investigate the interaction of ZNS with the CA isozymes involved in lipogenesis and other metabolic processes, through the crystallographic analysis. Here we report the X-ray crystal structure of the complex ZNS-hCA II at a resolution of 1.70 Å, showing that the ZNS participates in the classical inhibitory interactions with the Zn(II) ion and with specific residues in the active site of the hCA II.

Thus the activity of this drug in different metabolic pathways must be reconsidered also according to its possibility of interaction with different CAs.

[1] Leppik I. E. , *Seizure*, 2004, **13(1)**, S5-9. [2] Elan Pharmaceuticals WO03092682, **2003**. [3] Casini A., Antel J., Abbate F., Scozzafava A., David S., Waldeck H., Schafer S., Supuran C. T., *Bioorg. Med. Chem. Lett.*, 2003, **13**, 841-5.

Keywords: drug design, sulfonamides, carbonic anhydrase

P.04.15.5

Acta Cryst. (2005). A**61**, C242

Structures of 5-methylthioribose Kinase: Catalytic Mechanism and Drug Design

Shao-Yang Ku[a,b], Patrick Yip[b], Kenneth A. Cornell[c], Michael K. Riscoe[d], P. Lynne Howell[a,b], [a]*Department of Biochemistry, University of Toronto.* [b]*Hospital for Sick Children, Toronto, Canada.* [c]*Department of Chemistry, Boise State University, Boise, USA.* [d]*Department of Chemistry, Portland State University, Portland, USA.* E-mail: syku@sickkids.ca

The essential amino acid methionine plays critical roles in a variety of cellular functions but is energetically costly to synthesize. As a consequence, pathways to salvage methionine have evolved in almost all organisms. 5-methylthioribose (MTR) kinase is a key enzyme in this pathway in microorganisms and certain plants, and the absence of a mammalian homolog suggests that the enzyme is a good target for the design of novel antibiotics against MTR kinase containing pathogens and selective herbicides. Recombinant *B. subtilis* MTR kinase has been expressed, purified and crystallized with the detergent CHAPS, and structures of the apo enzyme, ADP, ATP and ATP-MTR complexes have been determined. The first structure was determined by MAD technique using holmium in complex with ADP as the phasing derivative. The structure of MTR kinase has a eukaryotic protein kinase fold, and is similar to 3',5'-aminoglycoside phosphotransferase and choline kinase. Structures of MTR kinase with and without its substrate reveal local conformational flexibility and illuminate a detailed catalytic mechanism of the enzyme. These structures also provide a blueprint for future structure or mechanism based drug design.

Keywords: protein crystallography drug design, methylthioribose kinase enzymatic mechanism, methionine recycing pathways

P.04.15.6

Acta Cryst. (2005). A**61**, C242

Dobexilate as a Lead Compound in Angiogenesis Inhibition Search

Israel Sánchez, Rosa Maria Lozano, Antonio Romero, Guillermo Gimenez, *Department of structure and protein function, CIB-CSIC, Madrid, Spain.* E-mail: israelsf@cib.csic.es

Fibroblast growth factors (FGFs) are powerful angiogenic polypeptides, whose mitogenic activity requires the presence of heparin-like compounds. Inhibition of angiogenesis-promoting factors such as fibroblast growth factor is considered to be a potential procedure for inhibiting solid tumor growth. Although several peptide-based inhibitors are currently under study, the development of antiangiogenic compounds of small molecular size is a pharmacological goal of considerable interest. We have study the effect of dobexilate *in vitro* and *in vivo* in order to find a minimum compound capable of inhibiting angiogenesis and tumor growth. Cell cultures as well as animal model experiments have shown clearly an angiogenesis suppressing effect event at low concentration as 50 μM. To provide structural information of this process we have solve the three-dimensional structure of a dobexilate-FGF complex. The structure gave us a clear image of the antiangiogesis mechanism of the dobexilate molecule which consists in the steric hindrance of interaction between the FGF molecule and the low affinity membrane receptor of this molecule in the plasma membrane, hampering in this way the beginning of the signalling cascade. Further studies of different groups in the minimal dobexilate structure could give us a more powerful and less toxic antiangiogenic compound using the disruption of the interaction of FGFs with heparin and heparan sulphates as its principal mechanism.

Keywords: FGFs, antiangiogenesis, dobexilate

P.04.15.7

Acta Cryst. (2005). A**61**, C242-C243

HIV Protease Inhibition Seen by X-ray Diffraction and Molecular Dynamics

Jindřich Hašek, Karel Zimmermann, Tereza Skálová, Jan Dohnálek, Jarmila Dušková, Hana Petroková, Eva Vondráčková, *Institute of Macromolecular Chemistry Academy of Sciences, CZ16206 Praha 6, Czech Republic.* E-mail: hasek@imc.cas.cz

Binding of inhibitor into the complex with HIV protease is accompanied by a large movement of protease flaps. X-ray crystallography shows the stable inhibited protease complexes [1], or unliganded proteases with "semi-opened" flaps and larger atomic displacement parameters. This work inspects conformational mobility