structure of human testis ACE in complex with a novel C-domain specific inhibitor, to 3.0 Å, which reveals detailed information on the interactions of this inhibitor with the active site.

In addition, we have carried out a normal mode analysis that reveals the intrinsic flexibility of tACE about its active site cleft. The intrinsic flexibility suggested by this study indicates a mechanism whereby subaccess could be achieved.

The information obtained in this study will be used in the design of new specific inhibitors of the C-domain of somatic ACE.

[1] Natesh R., Schwager S., Sturrock E., Acharya K., *Nature*, 2003, **421**, 551.

Keywords: glycoproteins, metalloprotein structures, drug targets

P.04.15.12

Acta Cryst. (2005). A**61**, C244

Structural Basis for Potent Inhibition of COX by Resveratrol-A Natural Product in Wine

Barbara Calamini[1], Bernie Santarsiero[1], Kiira Ratia[1], Michael Malkowski[2], John Pezzuto[3], Andrew Mesecar[1], [1]*Department of Medicinal Chemistry and Pharmacognosy and Center for Pharmaceutical Biotechnology, University of Illinois at Chicago.* [2]*Hauptman-Woodward Medical Research Institute, Purdue University.* [3]*Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University.* E-mail: bcalam1@uic.edu

Non-steroidal antiinflammatory drugs block the cyclooxygenase activity of prostaglandin-H synthase, also known as cyclooxygenase (COX), the enzyme that mediates biosynthesis of eicosanoids from arachidonic acid. Two enzyme isoforms have been identified: COX-1 which is constitutively expressed, and COX-2, which is inducible. Resveratrol (3,5,4′-trihydroxy-trans-stilbene) is a phytoalexin found predominantly in grapes and it has both antiinflammatory and cancer chemopreventive activity. One of the mechanisms of action of resveratrol is believed to be mediated through potent inhibition of COX-1 and COX-2 activity. We have determined the x-ray structure of COX-1 co-crystallized with resveratrol to 2.9 Å resolution using synchrotron radiation (BioCARS beamline 14-BM-C) to determine the binding mode of resveratrol in the active site. Using the crystal structures of COX-1/resveratrol and COX-2/flurbiprofen complexes, we performed computational docking studies of resveratrol and its two (3- and 4’-) sulfate metabolites using Dock 4.0.1. Our results indicate that the computed free energy values of binding for each of the docked resveratrol analogs are commensurate with their experimentally determined inhibition constants (K_i). However, the computational modeling results were unable to predict the selectivity in binding of resveratrol and its metabolites to the two enzymatic isoforms most probably due to the slight differences in binding affinities of these molecules for COX-1 or COX-2. This research is funded by grants from the National Cancer Institute (NIH: R03 CA92744-02 and 5 P01 CA48112-10).

Keywords: resveratrol, cyclooxygenase, docking

P.04.15.13

Acta Cryst. (2005). A**61**, C244

Structural Studies of Human α−thrombin

Helal Uddin Ahmed[1], Michele Cianci[2], Matthew Blakeley[3], Jarjis Habash[1], James Raftery[1], John R. Helliwell[1,2], Julia A. Hubbard[4], [1]*School of Chemistry, University of Manchester, Manchester, M13 9PL,UK.* [2]*CCLRC Daresbury Lab, Warrington, WA4 4AD, UK.* [3]*EMBL Grenoble Outstation, BP 156, 38042, Grenoble, Cedex 9, France.* [4]*Computational, Analytical & Structural Sciences, Glaxosmithkline, Gunnels Wood Road, Stevenage, Hertfordshire, SG1 2NY, UK.* E-mail: helal.u.ahmed@stud.man.ac.uk

Thrombin is a member of the serine proteinase family. The structure consists of two chains. Thrombin plays an important role in the coagulation of blood; contact with fibrinogen results in the formation of fibrin that polymerize into a blood clot.

X-ray data have been measured at ESRF ID 14.4, SRS MPW10 and at APS SBC. The four X-ray diffraction data sets achieved thus far are between 1.26 and 1.4 Å based on $<F/\sigma(F)> >2$ and completeness > 50%, two bound with hirugen only, and two with an inhibitor as well. We wish to identify hydrogen atoms at the active site and other key water hydrogens that are involved in the cleavage of fibrinogen.

In addition we wish to use neutron crystallography because the scattering factor for neutrons of deuterium equals that of C, N and O. We have so far grown a large (0.7x0.7x~0.3mm) thrombin crystal. Tests of the diffraction on the ILL LAue DIffractometer are imminent.

Overall, we plan to understand better how inhibitors bind to thrombin, so as to design enhanced drugs.

Keywords: thrombin, hydrogens, neutron diffraction

P.04.15.14

Acta Cryst. (2005). A**61**, C244

Active Structure of FR901451, a Potent Macrocyclic Elastase Inhibitor

Takayoshi Kinoshita[a], Tomoya Kitatani[b], Toshiji Tada[b], [a]*Exploratory Research Laboratories, Fujisawa Pharmaceutical Co. Ltd.* [b]*Research Institute for Advanced Science and Technology, Osaka Prefecture University, Japan.* E-mail: kinotk@v001.vaio.ne.jp

Bacteria produce a lot of bioactive and structurally unimaginable compounds. Among them, FR901451 from *Flexibaccter sp.* No.758 is known to have large tri-macrocyclic structure and to inhibit porcine pancreatic elastase, which in turn resembles the attractive drug target leukocyte elastase [1]. The crystal structure of FR901451 as bound to pancreatic elastase was solved at 2.5 Å resolution. The inhibitor occupies the most prominent subsites S1' to S3 of the elastase and prevents a hydrolytic attack by covering the active center with its rigid ring structure. The observed binding structure may help to design potent elastase inhibitors.

[1] Fujita T., Hatanaka H., Hayashi K., Shigematsu N., Takase S., Okamoto M., OkuharaM., *J. Antibiotics*, 1993, **47**, 1359.

Keywords: elastase inhibitor, macrocyclic compound, protein-inhibitor complex

P.04.15.15

Acta Cryst. (2005). A**61**, C244

Structure of Pteridine reductase (PTR1) from *Trypanosoma brucei*

Alice Dawson, P. Fyfe, F. Gibellini, K. McLuskey, N. Sienkiewicz, A. Fairlamb, W.N. Hunter, *School of Life Sciences, University of Dundee, Dow Street, Dundee DD1 5EH, UK.* E-mail: a.x.dawson@dundee.ac.uk

Anti-folate resistance in the trypanosomatid parasites is due in part to pterin reductase (PTR1) which is capable of reducing folate. This allows uptake of folate even when the primary enzyme, dihydrofolate reductase, is inhibited, and makes PTR1 an important drug target. The crystal structure of PTR1 from *Trypanosoma brucei* complexed with the cofactor NADPH and the inhibitor methotrexate has been determined to 2.2 Å. The protein structure is closely related to the previously determined *L. major* structure [1], with the cofactor and inhibitor bound in a similar fashion. The methotrexate molecule is significantly better defined in the *T. brucei* structure but there is no indication of increased MTX – protein interaction. A non-conservative Leu-Cys substition close to the active side is observed.

[1] Gourley D.G., et al., *Nature Str. Biol.*, 2000, **8**, 521-525.

Keywords: enzyme inhibitor design, biological macromolecules, folate dependent enzymes

P.04.15.16

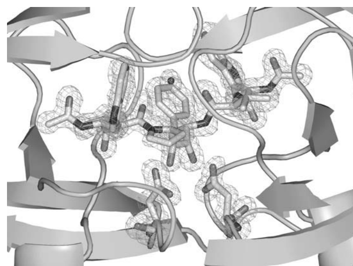
Acta Cryst. (2005). A**61**, C244-C245

Crystallography as a Tool to Identify the Best Inhibitor in a Complex Mixture

Nicola Demitri, Silvano Geremia, Lucio Randaccio, Jochen Wuerges Gianluca Tell, Fabio Benedetti, *Centre of Excellence in Biocrystallography, University of Trieste.* E-mail: geremia@univ.trieste.it

In this study we crystallized the HIV-1 aspartic protease using an equimolar mixture of four stereoisomeric inhibitors. Fourier maps obtained by high resolution diffraction data (up to 1.3 Å) from

synchrotron radiation, clearly show that the catalytic site is fully occupied by a single ordered molecule (see Figure). This permitted unambiguously the identification of nature and stereochemistry of the bound inhibitor. Furthermore, the clear electron density map, without residuals, suggests that the inhibition constant of this compound should be at least one order of magnitude lower than the constants of the other compounds. The full occupancy of the site indicates that its value is less than 1μM. This biocrystallographic study has allowed a first assessment of inhibition properties without the purification of the mixture and the classic activity assays that are normally conducted on each compound. The co-crystallization strategy could be applied in conjunction with combinatorial chemistry synthesis to discover, by self selection, new potent inhibitors.



Keywords: single-crystal structure analysis, inhibitor binding, isomers

P.04.15.17

Acta Cryst. (2005). A**61**, C245

Crystal Structure of a Disintegrin Heterodimer from *Echis carinatus* at 1.9 Å Resolution

Punit Kaur[a], Sameeta Bilgrami[a], Savita Yadav[a], A. S. Ethayathulla[a], R. Prem Kumar[a], Sujata Sharma[a], Markus Perbandt[b], Ch. Betzel[b], Tej P. Singh[a], [a]*Department of Biophysics, All India Institute of Medical Sciences, New Delhi-110029, India.* [b]*Institute of Medical Biochemistry and Molecular Biology, c/o DESY Notkestrasse 85, 22603, Hamburg, Germany.* E-mail: punit@aiims.ac.in

Disintegrins are a family of small proteins that bind to integrins specifically. Their binding site is characterized by the presence of Arg-Gly-Asp motif which indicates an RGD-dependant mode of interaction with integrins. The disintegrins interfere with the functions of integrins as antagonists. Disintegrin was isolated from the venom of *Echis carinatus* and crystallized in the tetragonal space group $P4_32_12$ with a=b=90.7Å and c=55.5Å. It exits as a heterodimer unlike the low resolution structure which existed as a homodimer with its two subunits related by a two fold crystallographic symmetry. It is interlinked by two disulfide bonds at the N-terminal region and contains 64 amino acid residues in each chain. Each monomer contains three pairs of six antiparallel β-strands and is stabilized by four disulphide bridges. It has been refined to an R-factor of 0.212 and R_{free} of 0.251 for all the data. The two chains of the dimer are anchored at N-terminal but diverge away at their C-termini exposing the Arg-Gly-Asp motif onto opposite directions, thus enhancing their binding efficiency. This is one of its unique features. The structural studies of disintegrins can provide a useful framework for the design of potent antagonists of integrins.

Keywords: disintegrin, heterodimer, drug design

P.04.15.18

Acta Cryst. (2005). A**61**, C245

Structure-assisted Design of Inhibitors Targeting Coronavirus Main Proteases

Haitao Yang[a], Weiqing Xie[b], Mark Bartlam[a], Xiaoyu Xue[a], Kailin Yang[a], Dawei Ma[b], Zihe Rao[a], [a]*Laboratory of Structural Biology, Tsinghua University, Beijing 100084, China.* [b]*State Key Laboratory of Bioorganic and Natural Products Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, 354 Fenglin Lu, Shanghai 200032, China.* E-mail: yanght@xtal.tsinghua.edu.cn

Coronaviruses (CoVs) are important etiologic agents of upper respiratory and digestive tract diseases in humans and animals; especially, the severe acute respiratory syndrome (SARS). The viruses are characterized with a highly complex cascade of proteolytic processing the replicative polyproteins to control viral gene expression and replication, which was predominantly mediated by the viral main proteinase (M^{pro}, also called $3CL^{pro}$), therefore, an attractive target for drug development[1].

A series of novel compounds with Michael receptor was designed according to the crystal structures of 3 coronaviruses M^{pro}s. The solved structures of SARS-CoV and porcine transmissible gastroenteritis virus (TGEV) M^{pro}s individually complexed with these compounds revealed that inhibitors possessing *α,β*-unsaturated ester combined with peptidyl-binding elements specific for CoV M^{pro}s undergo a nucleophilic addition of the protease's catalytic Cys, resulting in covalent-bond formation and irreversible inactivation of the viral proteases. One compound in this series has exhibited potent and extensive inhibition effect on 6 CoV M^{pro}s covering all 4 groups within genus *Coronavirus*. Meanwhile, the novel small molecules showed low micromolar concentration of EC_{50} for inhibition of viral replication and very low cell toxicity. We suppose further modification of these compounds assisted with structural information might lead to discover drug candidates against all CoV-associated diseases, including SARS.

[1] Yang H., Yang M., Ding Y., Liu Y., Lou Z., Sun L., Zhou Z., Ye S., Pang H., Gao G., Anand K., Bartlam M., Hilgenfeld R., Rao Z., *Proc. Natl. Acad. Sci. USA*, 2003, **100(23)**, 13190-13195.

Keywords: SARS, main protease, drug design

P.04.15.19

Acta Cryst. (2005). A**61**, C245

Structural Insights into the Substrate Binding Mechanism, Inhibition and Regulation of Pim-1

Judit É Debreczeni, Alexander Bullock, Frank von Delft, Stefan Knapp, *Structural Genomics Consortium, University of Oxford, Botnar Research Centre, OX3 7LD Oxford, UK.* E-mail: judit.debreczeni@sgc.ox.ac.uk

Pim-1 is a highly conserved cytoplasmic serine/threonine kinase that was first discovered as a preferential proviral insertion site in Moloney Murine Leukemia Virus (MoMuLV) induced T-cell lymphomas. The expression pattern of Pim-1 is widespread and the protein is over-expressed in a series of tumors but highest expression levels are found in cells of the hematopoietic and lymphoid system. Pim-1 phosphorylates a number of signal transduction proteins involved in the regulation of cell cycle, apoptosis, differentiation and proliferation.

We determined the structure of human Pim-1 in complex with an inhibitor of the bisindolyl maleimide (BIM) class as well as in ternary complex with its consensus peptide (pimtide) and BIM-1/AMPPNP that provides interesting insight into the substrate binding and inhibition of Pim-1 and suggests further applications of BIM-like compounds for treatment of leukaemia and other Pim-1 dependent cancer types.

Structural analysis of the monophosphorylated Pim-1 and auto-phosphorylation studies show that the human Pim-1 kinase activity is not influenced by auto-phosphorylation of activation loop residues. The N-terminus of Pim-1 has been shown to be important for several Pim interacting proteins, it is therefore likely that phosphorylation at Ser8 indicated by phosphorylation mapping plays a role in modulating these interactions.

Keywords: kinase structure, phosphorylation, drug design

P.04.15.20

Acta Cryst. (2005). A**61**, C245-C246

Interdomain Communication in HCV Polymerase Abolished by Small-Molecule Inhibitors

Stefania Di Marco, Cinzia Volpari, Licia Tomei, Sergio Altamura, Steven Harper, Frank Narjes, Uwe Koch, Michael Rowley, Raffaele De Francesco, Giovanni Migliaccio, Andrea Carfí, *Istituto di Ricerche di Biologia Molecolare, "I.R.B.M. P. Angeletti", Via Pontina Km 30.600, 00040, Pomezia (Rome), Italy.* E-mail: stefania_dimarco@merck.com

The hepatitis C virus (HCV) polymerase is required for replication of the viral genome and is a key target for therapeutic intervention against HCV. We have determined the crystal structures of the HCV polymerase complexed with two indole-based allosteric inhibitors at 2.3 Å and 2.4 Å resolution. The structures show that these inhibitors