inhibitor molecules. The changes observed in mutant complexes underscore the significance of flexibility of both the enzyme and inhibitor molecules for understanding resistance mechanisms and for designing second generation inhibitors.

PS04.12.12 COMPLEXES OF CALMODULIN WITH ANTAGONISTS. Zs. Böcskei, V. Harmat, D. Menyhárd, G. Náray-Szabó, Eötvös University of Sciences, Budapest, Hungary, B. Vértessy, J. Ovádi, Institute of Enzimology of the Hungarian Academy of Sciences, Budapest, Hungary

Calmodulin is believed to be the most important mediator of Ca^{2+}-dependent signaling in eucarytic cells and is thought to play an essetial role in processes like cell proliferation and growth. Calmodulin is therefore a target for certain drugs and consequently a target of drug design experiments.

The structures of calmodulin as well as its complexes with a number of substrates has received widespread attention recently. This is because calmodulin plays a regulatory role in a number of processes by transforming the value of the intracellular Ca^{2+} -ion concetration into a more structured information. If Ca^{2+} concentration increases than two hydrophobic binding pockets of calmodulin become exposed facilitating the binding of certain amphilphilic regulatory helices of at least 30 different proteins of high biological importance. Calmodulin mediated enzyme activation can be efficiently inhibited by a number of pharmacological agents (antipsychotics, antidepressants, muscle relaxants etc.). Crystal structure of TFP (a potent antipsychotic phenothiazine type drug, kind of a reference molecule on the area) with calmodulin is known from the literature[1]. This shows that due to the effect of the small molecular antagonist TFP, calmoduline undergoes a very similar conformational change to that it suffers when it binds regulatory oligopeptide pieces of proteins normally regulated by calmodulin.

We have recently shown that drugs fairly different form TFP result in very similar calmodulin conformational changes. Furthermore we have also demonstrated that at low, physiologically relevant TFP concentrations the binding of the second TFP occurs in the C-terminal domain of calmodulin, unlike it was proposed earlier. A quaterner complex of calmoduline with Ca^{2+} and two different type of drugs has also been investigated resulting in some new insights into the inactivation of calmodulin.

[1] Vandonselaar, M., Hickie, R.A., Quail, J.W., Delbaere, L.T.J. (1994) Nature Structural Biology 1 795-801.

PS04.12.13 STRUCTURE ANALYSIS OF KEY DRUG DESIGN TARGET ENZYMES FROM HUMAN PATHOGENS. Christopher M. Bruns[1], Andrew S. Arvai[1], Andrew J. Nowalk[2], Timothy A. Mietzner[2], Duncan E. McRee[1], and John A. Tainer[1]. [1]Department of Molecular Biology, The Scripps Research Institute, 10666 N. Torrey Pines Rd., La Jolla, CA USA 92037; [2]Department of Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine, Pittsburgh, PA USA 15261

Two proteins that represent promising drug design targets against human pathogens are the major ferric iron binding protein (FBP) from *Neisseria* and *Haemophilus*, and glutathione-S-transferase (GST) from *Schistosoma*. These proteins are both members of structural families for which several crystal structures are already known, permitting rational design of inhibitors specific for particular family members.

FBPs from two species of pathogenic bacteria have been crystallized. FBP crystals from *Haemophilus influenzae* (which causes many infections, including meningitis) diffract X-rays to 1.6 Å resolution. Anomalous scattering from these crystals unambiguously reveals the position of the iron atom at the active site. Crystals of FBP from *Neisseria gonorrhoeae* (which causes gonorrhea) diffract to 2.8 Å. Crystallographic determination of these protein structures is underway.

The crystal structure of GST from *Schistosoma japonicum* has previously been determined, both in native form and in complex with praziquantel, the leading drug used to treat schistosomiasis (McTigue et al 1995). We are working to evaluate other potential inhibitors by X-ray crystallography.

Difficult sequence and structure alignment problems encountered during the analysis of these two families of proteins have motivated the development of a general purpose sequence alignment program designed to incorporate tertiary structure information into traditional sequence alignment methods. The progress of these studies will be discussed.

(1) McTigue, M;Williams, D; and Tainer, J (1995) *J.Mol.Biol.* **246**:21-27

PS04.12.14 THE STRUCTURE OF A SELF-ASSOCIATED COMPLEX OF STROMELYSIN. Garold L. Bryant, Jr., Eric T. Baldwin, Laura C. Kelley & Barry C. Finzel, Structural, Analytical & Medicinal Chemistry, Pharmacia & Upjohn, Inc., Kalamazoo, MI 49007

The matrix metalloproteinase stromelysin (MMP-3) has been the subject of intensive structural studies because of the apparent role of this class of enzymes in chronic inflammation and tumor progression. We report the 1.9 Å structure of an orthorhombic crystal form of the stromelysin catalytic domain (83-255) with two molecules in the asymmetric unit. In this form, the C-terminal residues of one molecule are bound in the P1-P3 subsites of the second molecule, with the carboxylate of Thr^{255} coordinated to the catalytic zinc. Most previously published peptidic inhibitors of both stromelysin and collagenase have bound toward the P' side of the substrate binding cleft. This self-complex reveals features of peptide binding on the P side. The substrate binding cleft is much wider on this side, and the bound peptide is found to lie along one edge of the cleft, making hydrogen bonds to the outermost strand (βIV) of the beta sheet that dominates the MMP folding topology. The S1 (Thr^{255}) and S2 (Glu^{254}) sidechains make no specific interactions with the enzyme, but the sidechain of S3 (Pro^{253}) is nestled into a strongly hydrophobic P3 specificity pocket formed by a juxtaposition of aromatic protein side chains (Tyr^{155}, His^{166}, Tyr^{168}). In the absence of any inhibitor occupying the P1'-P3' sites, a surrounding portion of the stromelysin structure possesses considerable flexibility.

PS04.12.15 RATIONAL DESIGN OF SULFONAMIDE INHIBITOR SPECIFIC FOR HUMAN CARBONIC ANHYDRASE I ISOZYME. Sugoto Chakravarty, Sunil Ghose, A. Bannerjee, K. K. Kannan, Bhabha Atomic Research Centre, Bombay - 400 085, India

Rational design of N - unsubstituted sulfonamide drugs which inhibit specifically a particular human carbonic anhydrase isozyme is of immense importance. From the refined crystal structures of human carbonic anhydrase I (HCAI) - sulfonamide complexes and subsequent molecular dynamics simulations, we have proposed a new sulfonamide inhibitor with stronger inhibition against HCAI. From the 2Å refined structures of three heterocyclic and aromatic sulfonamides complexed to HCAI the active site loop of L198, T199 and H200 was identified to be important for binding of the drug molecules (Chakravarty & Kannan, (1994). J. Mol. Biol., **243**, 298 - 309). The general features of binding of sulfonamides to HCAI were also revealed. The components of interaction energy which correlate well with the known inhibition constants for six sulfonamide complexes of both HCAI and HCAII were then

obtained using molecular dynamics simulations of XPLOR (Chakravarty, (1995). Ph.D. thesis, University of Bombay, India). Further simulations on nineteen other sulfonamide complexes whose crystal structures were not known, clearly revealed that the loop region comprising of L198, T199, H200, P201 and P202 were crucial for the design of HCAI - specific sulfonamide inhibitors. Several substituted aromatic and benzene sulfonamides were then docked into the active sites of the isozymes to optimise the interactions with these loop residues. Stereospecific substitution of methyl imidazole group in benzene sulfonamide resulted in strong interactions between the imidazole groups of the inhibitor and His 200 as observed from the energy minimised structure of the complex. Since His 200 is non - conserved between HCAI and HCAII, this indicated that the inhibitor would be more specific against HCAI. Energy minimisation of the resultant complex confirmed it. Further substitution of an alkyl chain resulted in additional stable non - bonded interactions with another non conserved active site residue Ala / Val 121. The compound BARCZM1 has been synthesised (Ghosh et al.; To be published) and is being characterized for its inhibitory properties the details of which will be presented.

PS04.12.16 THE CRYSTAL STRUCTURE OF HUMAN α-THROMBIN/ LY178550 COMPLEX: 5-AMIDINOINDOLE-4-BENZYLPIPERIDINE NON PEPTIDAL ACTIVE SITE INHIBITOR. Nickolay Y. Chirgadze, Daniel J. Sall, Robert Hermann, David K. Clawson, V. Joe Klimkowski, Gerald F. Smith, Donetta S.Gifford- Moore, William J Coffman, Eli Lilly and Company, Indianapolis, IN USA

Thromboembolic diseases remain a leading cause of mortality and morbidity in developed societies. Thrombin, a trypsin-like serine protease, is a key mediator in such disease states, primarily through fibrin formation and platelet aggregation.[1] In response to the well documented liabilities associated with warfarin,[2] an industry wide search has been initiated to discover safe and effective, orally active thrombin inhibitors that can be used to treat thrombotic disorders. Over the past few years, a number of very potent and selective inhibitors of thrombin have identified based on the NAPAP, Argatroban (MD-805), or a D-Phe-Pro-Arg structural motifs.[3] In general, however, the peptidal nature of these class of agents is prohibitive of high oral bioavailability.

In an effort to identify non peptidal inhibitors of thrombin which might have a more favorable pharmacokinetic profile than their peptide-related counterparts, we have prepared LY178550 as an initial lead for future structure-based drug design studies. Agent LY178550 consists of two primary components: 1) 5-amidinoindole which has been previously employed as an arginine surrogate in the design of inhibitors of arginine endopeptidases,[4] and 2) a hydrophobic 4-benzylpiperidine tail which has the potential to interact with the well characterized P_3 pocket of the thrombin active site.

A crystal structure of human α-thrombin complexed with LY178550 was determined by X-ray technique at 2.2 Å resolution. A final complex model has crystallographic R-factor of 14.4% with standard deviation from ideal for bond distances of 0.014 Å. A clear well defined electron density was observed for the inhibitor molecule in the active site. The inhibitor main chain has a L-shape and mimics conformation of arginal trypeptides[5]. This poster will describe the X-ray crystallographic study of the interaction of LY178550 with the active site of human α-thrombin.

[1] In *The Thrombin;* 1st ed.; Machovich, R., Ed.; CRC Press Inc.:, 1984.; V1, pp 122.
[2] Smith, G. F. et al. *Thromb. Res.* 1988, *50*, 163-174.
[3] Scarborough, R. M., *Ann. Rep. Med. Chem.* **1995**, *30*, 71.
[4] Geratz, J. D. *et al. Arch. Biochem. Biophys.* **1979**, 197, 551-559.
[5] Chirgadze, N.Y., *et al.,* Amer Crystallog. Ass Meeting, Aug 9-14, **1992**, v.20., 116.

PS04.12.17 INFLUENZA B/LEE/40 NEURAMINIDASE: X-RAY STRUCTURE OF ENZYME COMPLEXED WITH 4-GUANIDINO-Neu5ac2en. N. Y. Chirgadze, J. M. Colacino, K. A. Staschke, K. Briner, W. J. Hornback, J. E. Munroe, R. Loncharich, W. G. Laver*, Lilly Research Laboratories, Indianapolis, IN. USA, *The Australian National University, Canberra, Australia

Neuraminidase from influenza B/Lee/40 was crystallized and complexed with the potent and selective influenza neuraminidase inhibitor, 4-guanidino-Neu5Ac2en[1], by soaking the crystal in a concentrated solution of the inhibitor. Crystals suitable for X-ray have been obtained from PEG. They belong to tetragonal $P42_12$ space group containing one subunit per asymmetric unit. The enzyme-inhibitor complex crystal structure was determined by X-ray technique. An experimental data has been collected up to 2.8 Å resolution with an R_{merge} Of 10.4%. The crystal structure has been refined using a molecular dynamic procedure to yield a current crystallographic R-factor of 16%. The electron density of the inhibitor in the active site is well-defined and interpretation of the electron density distribution reveals an interaction between the C-4 guanidinium moiety of the inhibitor with the glutamic acid at position 117 which lies within a pocket of the active site of the neuraminidase. Similar results have been obtained using influenza A N9 neuraminidase[2]. Computational techniques are being used to analyze the enzyme-inhibitor interaction in terms of H-bond strengths.

[1] von Itzstein M. et al. (1993) *Nature* 363:418-423.
[2] Varghese et al. (1995) *Protein Sci.* 4:1081-1087.

PS04.12.18 RESISTANCE OF INFLUENZA A AND B VIRUSES TO 4- GUANIDINO-Neu5Ac2en. N. Y. Chirgadze[1], J. M. Colacino[1], K. A. Staschke[1], A. Baxter[1], G. Air[2], A. Bansal[2], E. Garman[3], J. Tang[1], W. J. Hornback[1], J. E Munroe[1], W. G. Laver[4], [1]Lilly Research Laboratories, Indianapolis, USA, [2]University of Alabama, Birmingham, USA, [3]University of Oxford, UK, [4]Australian National University, Canberra, AU

The reassortant influenza viruses, A/NWS-G70c (N9 neuraminidase [NA]) and B/HK/8/73 (HG) (B/Lee/40 NA), were selected for resistance to 4-Guanidino-Neu5Ac2en (4-GuDANA). The NA of resistant viruses was >200-fold more resistant to 4-GuDANA than was the NA of parental viruses. Resistant A and B viruses displayed 5% and 0.5%, respectively, of the parental NA activity yet both were able to undergo multicycle replication in MDCK cells and grow to equal titer in embryonated eggs. The expression by these viruses of NA activity in MDCK cells over a 72 hour period was extremely low relative to that of parental viruses. Sequence analysis revealed a single mutation in the NA gene leading to the change of a conserved Glu 119 (N9 numbering) to Gly for both A and B viruses. Glu 119 lies in a pocket beneath the active site of the enzyme and has been shown to interact with the C-4 guanidinium moiety of 4-GuDANA. The NA from 4- GuDANAr A/NWS-G70c has been crystallized. Although these crystals grew to only 0.2 mm in the largest dimension, data from low temperature (100K) X-ray diffraction experiments were collected with a merging Rvalue on intensities of 6.2% to 2.0 Å resolution. These data revealed the absence of the glutamate residue at amino acid position 119. We have been unable to obtain NA crystals of X-ray diffraction quality from the 4- GuDANAr B virus. In addition to the above viruses, wild-type influenza B/Lee/40 was selected for resistance to 4-GuDANA and the identical nucleotide change leading to the Glu to Gly alteration in the NA was found. Attempts to select a reassortant N2 virus (A/NWS-Tokyo) for resistance to 4- GuDANA have so far been unsuccessful. In preliminary experiments, 4- GuDANAr A/NWS-G70c was able to induce pyrexia in ferrets indicating that viruses with low levels of an altered NA retain pathogenicity, at least in this model of infection.