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