
Trypsin and N-[3-[4-(amidinophenoxy)carbonyl]phenyl]-2-methyl-2-propenoyl-N-allylglycine methanesulfonate (1), a new inhibitor to bovine trypsin complex provides the structural basis for the reaction mechanism of 1. Based on the present x-ray results, it is proposed that the potent inhibitory activity of 1 is mainly due to the formation of an acylated trypsin and its low rate of deacylation through an inverse-substrate mechanism.

References


PS04.12.28 REFINED CRYSTAL STRUCTURE OF BOVINE SPLEEN PURINE NUCLEOSIDE PHOSPHORYLASE AT 1.6 Å RESOLUTION. Matthew J. Pugmire, Chen Mao, Steven E. Elalick, Department of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York 14853

The low temperature X-ray crystal structure of bovine spleen purine nucleoside phosphorylase has been solved by molecular replacement and refined to high resolution in the native form and in complex with 9-deazainosine. Purine nucleoside phosphorylase (PNP) catalyzes the reversible phosphorylation of ribonucleosides and 2-deoxyribonucleosides of guanine and hypoxanthine. This phosphorylation is an important step in both the synthetic and metabolic pathways of nucleotides. PNP has become an important target for drug design due to its relationship with various immunological diseases. Refinement and rebuilding cycles were carried out using the programs XPLOR, CHAIN, and O. The native structure has been refined to an R-factor of 18.8 and a free-R-factor of 23.4 while the complex form has been refined to an R-factor of 20.0 and a free-R-factor of 24.8. The stereochemistry of the substrate is well defined at this high resolution as an important structural feature of the enzyme. The nucleoside shows an anti conformation of the glycosidic bond and C4'-endo - O4' exo puckering of the sugar ring. In the native crystal structure a Mg2+-H2O complex has been modeled between packing units while the native and complex forms show what appears to be a metal (possibly a first row transition metal) complex with 3 His residues and 2 water molecules that is on a 3-fold symmetry axis between packing units. Differences between the native and complex structures show that binding of substrate induces structural changes as a disordered loop becomes more ordered in an α-helix. These high resolution structures provide a detailed structural model of the PNP enzyme and aid in describing a possible reaction mechanism. Detailed structural information is also invaluable as inhibitors of PNP are designed as potential therapeutic agents.

PS04.12.29 THE CRYSTAL STRUCTURE OF α-THROMBIN WITH A NEW TYPE OF INHIBITOR: AERUGINOSIN 298-A. J. L. Rios Steiner, A. Tulinsky, Dept. of Chemistry, Michigan State University, E. Lansing MI 48824

Thrombin is a serine protease that plays different and important roles in the blood coagulation process. One of its most important functions is to convert fibrinogen to fibrin, the major component of a blood clot. It is one of the most studied targets towards the development and design for new thrombotic and antithrombotic drugs.

Aeruginosin 298-A (C30H48O7N6) is a unique tetrapeptide isolated from the fresh water blue-green alga Microcystis aeruginosa. Aeruginosin exhibits inhibition selectivity for thrombin and trypsin (IC50 of 0.3 mg/mL and 1.0 mg/mL, respectively) but displays no inhibition towards papain, chymotrypsin, elastase or plasmin. Crystallographic data for its complex with hirugen-thrombin were collected to 2.1 Å resolution. The ternary complex crystallizes in the monoclinic system C2, where a=71.97, b=72.48, c=72.24 Å, β=100.9°. The structure refinement near completion has R=15.6% and wR=17.3%.

This inhibitor binds to the catalytic site of thrombin in a D-PheProArg chloromethylketone-like fashion, where the arginyl group is fixed into the S1 site by the Asp189 and the carboxylic hydroxy group occupies the S2 site flanked by His57, Tyr60A and Trp60D. The P3 and the P4 sites are occupied by a L-Leu and a 4-hydroxyphenyllactic acid group, respectively. These sites are well positioned mainly due to the hydrogen bonding network present between the carboxyl and NH groups of Gly216 with the amide and carboxyl groups of the P3 and P2 residues coupled with a H-bond between the terminal hydroxyl of the P4 residue and the amide group of Gly219.

Crystals were prepared using diffusion techniques. The structure was solved using molecular replacement techniques (XPLOR) and refined using the PROLSQ program.

We would like to thank Dr. M. Murakami and Professor N. Pusenl, University of Tokyo, for providing a sample of the Aeruginosin 298-A.

PS04.12.30 CHARACTERIZATION OF A NON-COMPETITIVE INHIBITOR OF HUMAN GLUTATHIONE REDUCTASE. Savvas N. Savvides and P. Andrew Karpas, Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, NY 14853, USA

We have determined the crystal structure of a complex between human glutathione reductase (hGR) and its noncompetitive inhibitor 6-hydroxy-3-oxo-3Hxanthen-9-propionic acid (XAN) (Kis=27 mM, Ki=48 mM with respect to oxidized glutathione (GSSG), and Kis=144 mM, Kii=176 mM with respect to NADPH). The structure has been refined to an R-factor of 0.158 at 2.0 Å resolution, and reveals XAN bound in the large cavity present at the hGR dimer interface where it does not overlap the glutathione binding site. The inhibitor causes extensive structural changes at its binding site that primarily involve amino acid residues from a thirty residue α-helix which lines the cavity and contributes to the active site of hGR. Despite the lack of physical overlap of XAN with the GSSG binding site, no GSSG binding is seen in souls carried out with high XAN and GSSG concentrations, suggesting that some subtle interaction between the sites exists. An earlier crystallographic analysis on the complex between hGR and 3,7-diamo-no-2,8-dimethyl-5-phenyl-phenazinium chloride (safranin)
showed that safranin bound at this same site. We have found that
safranin also inhibits hGR in a noncompetitive fashion but it binds
about 16 times less tightly (Ks=453 mM, Ki=586 mM with re­
spect to GSSG) than XAN, and does not preclude the binding of
GSSG to the crystal structure. Our results were bolstered by crystallographic
results pertaining to the interactions XAN makes with the en­
zyme, the inhibition mechanism remains unclear. Although in structure­
based drug design competitive inhibitors are usually targeted,
XAN’s binding to a well defined site in hGR that is not well con­
served among homologs of hGR suggests that noncompetitive in­
hibitors could also serve as lead compounds for structure-based
drug design, in particular as components of chimeric inhibitors.

PS04.12.31 STRUCTURE AND INHIBITION OF
PLASMPSIN II, A HEMOGLOBIN-DEGRADING
ENZYME FROM P. FALCIPARUM. A. M. Silva*, A. Y. Lee1, S. V. Gulnik1, P. Mayer, J. Collins1, T. N. Bhat1, P. J. Collins1, R. E. Cachau1, K. E. Luker2, I. Y. Gluzman5, S. E. Francis5, A. Oksman2, D. E. Goldberg2 and J. W. Erickson1 (1) Structural Biochemistry Program, National Cancer Institute/SAIC, Frederick, Maryland 21702, USA. (2) Howard Hughes Medical Institute, Departments of Molecular Microbiology and Medicine, Washington University School of Medicine, St Louis, Missouri 63110, USA.

Plasmodium falciparum is the major causative agent of malar­
ia, a disease of worldwide importance. Resistance to current drugs such as chloroquine and mefloquine is spreading at an alarming rate and our antimalarial armamentarium is almost de­
pleted. The malarial parasite encodes two homologous aspartic
proteases- plasmepsins I and II- which are essential components
of hirugen-inactivated thrombin (space group C2) in 2 - 7 mM
PEG-400.12.32 CRYSTAL STRUCTURES OF TWO THROM­
BIN COMPLEXES WITH NOVEL PEPTIDE MIMETIC IN­
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Thrombin is a trypsin-like blood serine protease specialized
in catalyzing the proteolysis of fibrinogen to fibrin monomers dur­
ing the final stage of the coagulation cascade. Synthetic active site
inhibitors of thrombin represent potentially useful antithrom­
botic agents and are showing promise as alternatives to current heparin­
based therapeutics.

The crystal structures of human α-thrombin complexed sep­
arately to two similar peptidomimetic active site inhibitors (MOL124-1 and MOL126-1) have been solved to 2.1 Å res­olution and refined using restrained least squares with R-factors less
than 0.18. Ternary complexes were prepared by soaking crystals of
hirugen-inactivated athrombin (space group C2) in 2 - 7 mM
solutions of each synthetic inhibitor over a period of 5 days. Both
inhibitors resemble D-Phe-Pro-Arg chloromethylketone (PPACK)
in structure and bind in a similar fashion within the catalytic site.
As with other active site inhibitors of thrombin, the arginy1 side
chaining of the P1 residue of each inhibitor is bound within the pri­
mary specificity pocket of the protein making salt-bridged hydro­
gen bonds with the acidic side chain of Asp 189. Moreover, this
garginine residue forms an expected hemiketal with Ser 195 in both
complexes. The P2 residue of each mimetic possesses a novel bi­
cyclic structure that constrains the precleavage portion of the in­
hibitor in an extended, substrate-like conformation, and interacts
with the S2 site much like the proline residue of thrombin-bound
PPACK. An aromatic P5 residue, D-phenylalanine, occupies the
aryl binding site in both complexes. In MOL124-1, this residue is
acylated at the amino-terminus, and makes several stabilizing in­
teractions with the protein. One of the two inhibitors (MOL126-1)
possesses an aromatic, α-ketoamide-linked P1’ residue that is
hydrogenbonded to His 57 and makes numerous apolar intera­
tions within the $1’ and S2’ sites of thrombin. This residue, absent
in MOL124-1, is responsible for enhanced inhibitory activity.

PS04.12.33 STRUCTURE BASED DISCOVERY OF A NEW
CLASS OF ENZYME INHIBITORS. Thomas J. Stout*, M.
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Thymidylate Synthase (TS) is an important target for the de­
velopment of anti-cancer therapeutics. TS forms a biosynthetic
template for the production of the DNA nucleotide thymidine
(T) by catalyzing the reductive methylation of dUMP to dTMP.
As such, many inhibitors of TS have been developed as mecha­
nism based substrate or co-factor analogues, but currently less than five have been approved for use as anti-cancer therapeutics. We
have used structure-based modeling methods and single-crystal
X-ray crystallography to discover an entirely new series of TS
inhibitors unrelated to substrate or co-factor (1). First generation
leads have subsequently been sequentially improved through cy­
cycles of design, synthesis and crystallography to yield TS inhib­
itors of improved potency and specificity.

These phthalid-based compounds display a new mode of gen­
eral enzyme inhibition through non-specific occlusion. The crys­
tal structures show multiple modes of ligand binding which vary
significantly with small modifications to the ligands. In addition, kinetic analysis shows concentration dependent inhibition, implic­
itating a non-specific mode of binding. Indeed, the hydrophobic
non-specific interactions found within the crystal structures ap­
pear to predominate over potential hydrogen bonds. The com­
bined occupied volumes of five different enzyme-inhibitor com­
plexes map out a new ligand-accessible space which can now be
used for future structure-based design of inhibitors with addi­
tional binding specificity.