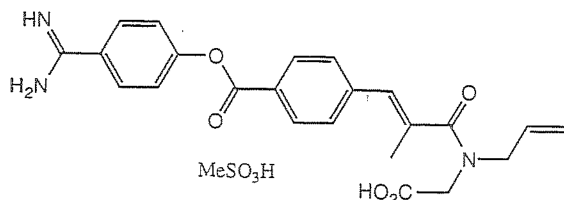
PS04.12.27 UNIQUE BINDING OF A NOVEL SYNTHETIC INHIBITOR TO BOVINE TRYPSIN. Yoshihiko Odagaki,+Hisao Nakai,+Kazuhiko Senokuchi, +Masanori Kawamura,+Nobuyuki Hamanaka,+Masahiro Nakamura,§Koji Tomoo§ and Toshimasa Ishida§ +Ono Pharmaceutical Co., Ltd. §Osaka University of Pharmaceutical Sciences

Trypsin and N-[3-[4-[4-(amidinophenoxy)carbonyl] phenyl]-2-methyl-2propenoyl]-*N*-allylglycine methanesulfonate (**1**), a newly designed and orally active synthetic trypsin inhibitor, were cocrystallized. The space group of the crystal is $P2_12_12_1$ with cell constants a = 63.74 Å, b = 63.08 Å and c =69.38 Å, nearly identical to that of the orthorhombic crystal of guanidinobenzoyl-trypsin. The structure was solved by molecular replacement and refined to a crystallographic residual R = 0.176. The refined model of the **1**-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.



N-[3-[4-[4-(amidinophenoxy)-carbonyl]phenyl]-2-methyl-2-propenoyl]-*N*-allylglycine methanesulfonate **1**

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PS04.12.28 REFINED CRYSTAL STRUCTURE OF BOVINE SPLEEN PURINE NUCLEOSIDE PHOSPHORYLASE AT 1.6 Å RESOLUTION. Matthew J. Pugmire, Chen Mao, Steven E. Ealick, 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 phosphorolysis of ribonucleosides and 2'-deoxyribonucleosides of guanine and hypoxanthine. This phosphorolysis is an important step in both the synthetic and metabolic pathways of nucleosides. 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 are other important structural features 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 $Mg\text{-}6H_2O$ complex has been modeled between packing units while both 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 ($C_{30}H_{48}O_7N_6$) is a unique tetrapeptide isolated from the fresh water blue-green alga *Microscytis aeruginosa.* Aeruginosin exhibits inhibition selectivity for thrombin and trypsin (IC_{50} 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 2.1Å resolution. The ternary complex crystallizes in the monoclinic system C2, where a=71.97, b=72.48, c=72.24Å, ß=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 argininol group is fixed into the S1 site by the Asp189 and the carboxy-6-hydroxy octahydroindole group occupies the S2 site flanked by His57, Tyr60A and Trp60D. The P3 and the P4 sites are occupied by an *L*-Leu and a 4-hydroxyphenyllactic acid group, respectively. These sites are well positioned mainly due to the hydrogen bonding network present between the carbonyl and NH groups of Gly216 with the amide and carbonyl 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. Fusetani, 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 Karplus, 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-3oxo-3Hxanthene-9-propionic acid (XAN) [Kis=27 mM, Kii=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 a-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 soaks 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-diamino-2,8-dimethyl-5-phenyl-phenazinium chloride (safranin)