PS04.01.37 THE CRYSTAL STRUCTURE OF A BI-FUNCTIONAL KUNITZ TYPE CYSTEINE PROTEINASE INHIBITOR AT 1.9Å RESOLUTION. Minakshi Ghosh1, Michael Mares2, Karl Harlos1 and Colin Blake1. 1Laboratory of Molecular Biophysics, Rex Richards Building, South Parks Road, Oxford OX1 3QU, UK; 2Institute of Organic Chemistry and Biochemistry, CSAV, Flemingovo namesti 2, Prague, Czech Republic.

The cysteine proteinase inhibitor from potato (PCI) belongs to the Kunitz-type soybean trypsin inhibitor (STI) family and shows inhibitory properties against a broad range of serine proteinases too. It consists of 180 amino acid residues. The primary sequence of PCI shows about 20% sequence homology with the other proteinase inhibitors of the family.

The crystal structure of PCI has been determined to 1.9Å by the method of single isomorphous replacement and refined using the program XPLOR. The current model includes 179 amino acid residues and 107 water molecules, with a rms deviation of 0.008Å in bond lengths and 1.6° in bond angles from identity. The crystallographic R-factor is 21.1% (Free R-factor = 25.6%) including all reflections from 8Å-1.9Å. The three-dimensional structure of PCI shows the characteristic β-trefoil fold of the STI Kunitz inhibitor family. The core of the structure, consisting of 12 antiparallel β-sheets are similar to ETI, the trypsin inhibitor from *Erythrina caffra* seeds, while the long loops connecting the β-strands are considerably different in length and fold. The reactive sites of the other inhibitors for trypsin and those of a bi-functional inhibitor of proteinase K (which is similar to the bacterial subtilisin) and α-amylase, belonging to the same family, are all seen to be located on these loops. The position of the active site of PCI is not known yet but it is likely that this is also located on one of these loops. Identification of the reaction centres is in progress and is expected to reveal how, on a common structural framework, different reaction centres for different proteinases are accommodated.

PS04.01.38 STRUCTURE OF EQUINE INFECTIOUS ANEMIA VIRUS PROTEINASE COMPLEXED WITH AN INHIBITOR. Jukka Kervinen (1), Alla Gustchina (1), David Powell (2), Alexander Zdanov (1), John Kay (2), and Alexander Wlodawer (1), (1) Macromolecular Structure Laboratory, NCI-Frederick Cancer Research and Development Center, ABL-Basic Research Program, Frederick, MD 21702, USA, (2) University of Wales, Cardiff, Wales, UK

Equine infectious anemia virus (EIAV) belongs to the lentiviral family of retroviruses and it is a causative agent of an infectious anemia (swamp fever) in horses. EIAV proteinase (PR) processes viral polyproteins into functional molecules. X-ray structure of a complex of recombinant EIAV PR with the inhibitor HBY-793 has been solved at 1.8 Å resolution and refined to a crystallographic R-factor of 0.136. The overall fold of EIAV PR is very similar to the fold of other retroviral proteinases. However, the appearance of the second α-helix in the monomer is a feature not previously reported for retroviral PRs. Despite their strong structural homology, different retroviral PRs show extreme diversity in the binding of substrates and inhibitors. The diversity in affinity may be explained by the structural differences caused by sequence diversity at critical positions in the active site cleft and nearby regions. Here, comparison of the high resolution structures of EIAV PR, feline immunodeficiency virus PR, HIV PR, and Rous sarcoma virus PR are used to explain some of those differences.

PS04.01.39 CRYSTAL STRUCTURE OF HUMAN PEPSINOGEN A. Katherine S. Bateman1, Maia N. Chernaia1, Nadya I. Tarasova2, Michael N. G. James1, 1MRC Group in Protein Structure and Function, Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada TOG 2H7, 2Molecular Aspects of Drug Design Section, ABL-Basic Research Program, National Cancer Institute, FCDRC, P. O. Box B, Frederick, Maryland 21702

The three dimensional structure of human pepsinogen A has been solved by the method of molecular replacement. The protein fold is similar to those of previously determined aspartic proteinase zymogens, porcine pepsinogen[1] and human progastricsin[2]. Two similar domains consisting mostly of ß-sheet make up the enzymatic portion of the zymogen. A smaller segment of two ß-strands and short α–helices sits between the two large domains in the active site cleft.

Pepsinogen is the inactive protein precursor of pepsin, an aspartic proteinase found in the gastric mucosa. A 46 amino acid prosegment is removed by autocleavage once pepsinogen has been exposed to acidic pH. Before enzyme activation, a portion of the prosegment occupies and blocks the binding cleft. The catalytic aspartic acid residues 32 and 215 are electostatically stabilized by residues Lys36p, Tyr37p and Tyr9, p denoting residues of the prosegment. A conformational rearrangement accompanies a pH decrease which then allows access to the binding site. Aspartic proteinases are critical in many metabolic pathways such as the regulation of blood pressure. Regulation of aspartic proteinases through drug design will be greatly assisted by an understanding of biological mechanisms for enzyme inhibition and control.

Crystals of human pepsinogen A were grown in space group $P2_12_12$ having unit cell dimensions a=91.6, b=105.2, c=40.2 Å with one molecule per asymmetric unit. High resolution data were collected at the Photon Factory in Tsukuba, Japan to 1.7 Å. The program AMORE[3] was used to solve the structure by molecular replacement with porcine pepsinogen A[1] as the replacement model. The model is currently being refined; the R-factor and R-free of the present model being 21.6% and 29.7% respectively from 20 to 2 Å.

1.Sielecki, A. R., *et al.* J. Mol. Biol. 219, 671. (1991).
2.Moore, S. A., *et al.* J. Mol. Biol. 247,466. (1995).
3.Navaza, J. Acta Cryst. A50, 157. (1994).

PS04.01.40 CRYSTAL STRUCTURE OF HUMAN SALIVARY CYSTATIN. Narayanan Ramasubbu, Michael J. Levine, Department of Oral Biology and Dental Research Institute, School of Dental Medicine, State University of New York at Buffalo, Buffalo, NY 14214, USA

Human saliva contains several isoforms of thiol proteinase inhibitors known as cystatins which have potential antimicrobial and antiviral functions. These properties suggest an important role in maintaining oral health. Salivary cystatins contain 121 amino acids and have a molecular weight of ~13,500 daltons. There is considerable differences in the ability of salivary cystatins to inhibit papain and cathepsins.In an effort to define the role of cystatins in the oral cavity, we have initiated a structure-function-mutagenesis approach. In this regard, we have expressed and purified several recombinant salivary cystatin variants using an Escherichia coli expression system, pGEX-2T. One of the variants (Δ12-16) lacks a five-residues (G-G-I-Y-N) near the N-terminus. Interestingly, this variant possesses higher thiol-proteinase inhibitory activity towards papain compared to the full-length recombinant cystatin.

Cystatin Δ12-16 was crystallized by sitting drop vapor diffusion method in a silica gel environment using a protein concentration of 30 mg/mL. Crystals are of space group P622 and the unit cell

constants of a = b = 85.41, c = 131.6 Å, $\alpha = \beta = 90°$, $\gamma = 120°$, with two molecules per asymmetric unit, Vm = 2.6 Å^3/Da. Native data were collected to a resolution of 2.5 Å using a MAR imaging plate mounted on a Rigaku rotating anode. The complete native data set contains 9740 ($I > 2\sigma(I)$; R_{merge} 5.1%; ~94% complete). The structure was solved using the chicken egg white cystatin structure as a starting model by molecular replacement technique and refined to an Rfactor of 25% using XPLOR. The refinement is still in progress. The details of the structure determination, refinement and its thiol proteinase activity will be described.

Work supported by USPHS grants DE10621 and DE08240.

PS04.01.41 CRYSTAL COMPLEXES OF WILD TYPE AND MUTANT CI-2 INHIBITOR WITH SUBTILISIN CARLSBERG: THE STRUCTURAL BASIS FOR BINDING KINETICS AND A HIGH RESOLUTION STUDY. Wojciech R. Rypniewski[1], Claus von der Osten[2], Torben Halkier[2] & Keith S. Wilson[1]. [1]European Molecular Biology Laboratory, c/o DESY, Notkestrasse 85, D-22603 Hamburg, Germany; [2]Novo Nordisk, Novo Alle, DK-2880 Bagsvaerd, Denmark.

Both wild type CI-2 and reactive loop mutant M59P form stable complexes with alcalase, a variant of subtilisin Carlsberg, but K_i values differ by 10^5. The crystal structures of both complexes were solved using the synchrotron radiation on the EMBL beamlines at the DORIS synchrotron in Hamburg and examined to determine the basis of the differences in binding kinetics. As expected, the differences can be derived from the special structural properties of proline, but at first glance this was not clear and a simplistic explanation had to be abandoned. A detailed explanation followed from examining the high resolution structures.

The wild type structure has been solved at atomic resolution (1Å). The high quality and resolution of the data allowed a detailed analysis of this 32 kdal protein complex, with refined anisotropic temperature factors, direct visualisation of most hydrogen atoms, unrestrained refinement of some stereochemical parameters including peptide bond planarity and chiral volumes, and details of water structure and its interactions with the protein.

PS04.01.42 STRUCTURE OF A SOLUBLE FORM OF THE KEX1P SERINE CARBOXYPEPTIDASE FROM SACCHAROMYCES CEREVISIAE. Brian H. Shilton, Yunge Li, Daniel Tessier, David Y. Thomas and Miroslaw Cygler Montreal Joint Centre for Structural Biology Biotechnology Research Institute, NRC, 6100 Royalmount Avenue Montreal, Quebec H4P 2R2

Kex1p, a prohormone processing enzyme from Saccharomyces cerevisiae, is a membrane-anchored serine carboxypeptidase involved in the maturation of alphapheromone and K1 killer toxin. Residues 23 to 506 represent the catalytic domain of Kex1p which exhibits sequence similarity to a number of other carboxypeptidases, including three for which crystal structures have been solved-wheat serine carboxypeptidase (CPDW-II; Liao et al., 1992, Biochemistry 31:9796-9812), yeast carboxypeptidase Y (CPD-Y; Endrizzi et al., 1994, Biochemistry 33:11106-11120), and human protective protein (Rudenko et al., 1995, Structure 3:1249-1259). Kex1p is unique among these proteins in that it is highly specific for lysyl or arginyl residues at the C-terminus of the substrate. We are interested in the functional differences between Kex1p and related carboxypeptidases, as well as the relationship between these enzymes and the larger alpha/beta hydrolase family.

Expression of a genetic construct coding for the catalytic domain of Kex1p yielded a soluble form of the enzyme (Kex1p-s; Mr = 56 kDa; Latchinian-Sadek and Thomas, 1994, Eur. J. Biochem, 219:647-652) which retains the kinetic characteristics of its membrane-bound parent. Kex1p-s has been crystallized in space group $P2_12_12_1$ with unit cell dimensions a=56.6 Å, b=84.0 Å, c=111.8 Å (Shilton et al., 1996, Protein Science, in the press); there is one molecule in the asymmetric unit. The structure has been solved by a combination of MIR and molecular replacement, and is currently under refinement against a 2.4 Å native data set collected at 120 K.

PS04.01.43 THE MOLECULAR STRUCTURE OF HUMAN CYTOMEGALOVIRUS ASSEMBLIN - A NOVEL SERINE PROTEASE AND AN UNIQUE DRUG TARGET Huey-Sheng Shieh, William C. Stallings, Ravi G. Kurumbail, Anna M. Stevens, Jina Y. Pak, Eric J. Sturman, Roderick A. Stegeman, Mark O. Palmier, Arthur J. Wittwer, and Barry C. Holwerda, Searle/Monsanto, BB4K, 700 Chesterfield Parkway N., Chesterfield, MO 63198, USA

Human cytomegalovirus (HCMV), a herpesvirus, is a ubiquitous opportunistic pathogen which causes clinically apparent disease in congenitally infected infants and in immunocompromised and immunosuppressed adults. All members of the herpesvirus family encode a unique serine protease, assemblin, which is necessary for viral replication. HCMV assemblin was crystallized in space group $P4_2$ with a dimer in each asymmetric unit. The crystals diffract to 2.2 Å resolution. The structure was determined at 2.8 Å resolution by multiple isomorphous replacement methods combined with solvent flattening and averaging. The assemblin molecule folds in a eight-stranded barrel which is stabilized by surrounding α helices. One of the exterior surfaces of the barrel contains the constellation of residues that forms the active site. This folding is quite different from the other classes of serine protease, such as the two-domain structures of chymotrypsin series, and the one-domain structures of subtilisin and serine carboxypeptidase II series. The primary feature of the dimer interface in the assemblin is the α helix (residues 217 235) that runs parallel to the dimer dyad and is recognized by a groove formed by three helices from the other subunit of the dimer. The residues around the putative internal cleavage sites (143 and 209) consistently show poor electron density in various Fourier maps and have not been modelled in the current structure. The structure has been used to identify residues that may be involved in catalysis and the role of these residues has been investigated by site directed mutagenesis. These results will also be presented.

PS04.01.44 CRYSTAL STRUCTURE OF THE COMPLEX OF PORCINE PANCREATIC TRYPSIN WITH KUNITZ-TYPE SOYBEAN TRYPSIN INHIBITOR. Hyun Kyu Song and SeWon Suh, Department of Chemistry and Center for Molecular Catalysis, Seoul National University, Seoul 151-742, Korea.

The crystal structure of the complex formed between the procine pancreatic trypsin and Kuntiz-type soybean trypsin inhibitor has been determined at 1.75 Å resolution (R-factor of 18.9 % for 31,038 unique reflections with $Fo > 2\sigma F$ in the range 8.0-1.75 Å). The root mean square deviations from ideal stereochemistry are 0.013 Å for bond lengths and 1.32° for bond angles. Moleculare replacement models for porcine pancreatic trypsin and Kunitz-type soybean trypsin inhibitor were porcine, ß-trypsin complexed with Momordica charantia trypsin inhibitor [Hunag *et al.* (1993) J. Mol. Biol. 229, 1022-1036, pdb ID code: lsmf] and Erythrina caffra trypsin inhibitor [Onesti et al. (1991) J. Mol. Biol. 217, 153-176, pdb ID code: 1tie], respectively. This study at high resolution significantly improved the model of the complex, previously determined at 2.6 Å resolution by multiple isomorphous replacement method [Sweet *et al.* (1974) Biochemistry 13, 4212-4228]. The improved resolution has enabled positioning of the protein atoms with greater accuracy as well as locating solvent atoms, including water molecules near the catalytic residues.