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