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.

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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.