PS04.07.24 A NEW SERINE PROTEASE FOLD REVEALED BY THE CRYSTAL STRUCTURE OF HUMAN CYTOMEGALOVIRUS PROTEASE. L. Tong[1], C. Qian[1], M.-J. Massariol[2], P.R. Bonneau[2], M.G. Cordingley[2], L. Lagacé[2], [1]Boehringer Ingelheim Pharmaceuticals, Inc., 900 Ridgebury Road, Ridgefield, CT 06877, [2]Bio-Méga/Boehringer Ingelheim Research, Inc., 2100 rue Cunard, Laval, Québec, Canada H7S 2G5

A new polypeptide backbone fold for serine proteases has been identified based on the crystal structure of human cytomegalovirus protease. The structure was determined at 2.5Å resolution by the multiple-wavelength anomalous diffraction technique using the seleno-methionyl protein and refined at 2.0Å resolution. It reveals a seven-stranded mostly-antiparallel ß-barrel, which is surrounded by seven helices. The active site residues (Ser-132 and His-63) are situated on the outside of the ß-barrel and in a groove on the surface of the protein. The structure suggests that the third member of the catalytic triad is probably His-157. The protease of herpesviruses plays an essential role in the production of infectious virions and is an attractive target for the developement of anti-herpes agents. The crystal structure information will help in the design and optimization of inhibitors against herpes virus protease.

[1] Tong, L., Qian, C., Massariol, M.-J., Bonneau, P.R., Cordingley, M.G. & Lagacé, L. *Nature*, submitted.

PS04.07.25 THE CRYSTAL STRUCTURE OF HUMAN CYTOMEGALOVIRUS PROTEASE. Ping Chen, Robert Almassy, Hideaki Tsuge*, Dave Matthews, Chris Pinko, Cindy Gribskov, and Chen-Chen Kan, Agouron Pharmaceuticals Inc., 3565 General Atomic s Court, San Diego, CA92121, *Japan Tobacco Inc., Pharmaceutical Division, Minato, Tokyo, Japan

Human cytomegalovirus (HCMV) is a beta herpes virus. HCMV, like all other members of the Herpes virus family, encodes a protease that is essential for capsid maturation and production of infectious virus. The catalytic domain of the HCMV protease was produced in E.coli as a single-chain protein and was crystallized in space group $C222_1$ with two dimers per asymmetric unit. The crystal structure was determined at 2.5Å resolution using the ISAS method and noncrystallographic symmetry averaging. Our current model has been refined against 2.3Å data collected on an image plate at -170°C. The HCMV protease structure has a new fold different from that of any other known protease. There is a central core comprising two orthogonal 4-stranded beta sheets surrounded by eight alpha-helices. Residues in three flexible surface loops, including two associated with internal cleavage sites at amino acids 143 and 209, have not been modeled into the current structure. Dimerization of HCMV protease is mediated primarily by burying four turns of alpha-helix (218-232) from one monomer into a pronounced depression in the surface of the other monomer. Only two of three residues previously implicated by amino acid sequence alignment and mutagenesis as particpating directly in catalysis (Ser-132 and His-63 but not Glu-122) are actually located in the active site. This novel structure is being used to further understand the catalytic mechanism, and to design inhibitors as potential anti- viral agents.

PS04.07.26 THE THREE-DIMENSIONAL STRUCTURE OF APOPAIN/CPP32, A KEY MEDIATOR OF APOPTOSIS. Jennifer Rotonda and Joseph W. Becker, Department of Biochemistry, Merck Research Laboratories, PO Box 2000, Rahway, New Jersey 07065

Cysteine proteases related to mammalian interleukin-1β converting enzyme (ICE) and to its *C. elegans* homologue, CED-3, play a critical role in the biochemical events that culminate in apoptosis. We have determined the three-dimensional structure of a complex of the human CED-3 homologue CPP32/apopain with a potent tetrapeptide-aldehyde inhibitor.[1] The protein resembles ICE in overall structure, but its S_4 subsite is strikingly different in size and chemical composition. These differences account for the variation in specificity between the ICE- and CED-3-related proteases and enable the design of specific inhibitors that can probe the physiological functions and disease states with which they are associated.

We have solved the three-dimensional structure of apopain in complex with the peptide-aldehyde inhibitor Ac-DEVD-CHO at a nominal resolution of 2.5 Å. The crystals belong to the orthorhombic space group I222 with a=69.81, b=84.62, c=96.79 Å with one enzyme:inhibitor complex per asymmetric unit. Three-dimensional diffraction data extending to a resolution of 2.5 Å were collected at room temperature using a Siemens area detector and CuKα radiation from a Rigaku RU-200 rotating-anode X-ray generator. 20,801 observations of 8,929 unique reflections were merged with an R-factor of 5.55%. The structure was solved by molecular replacement, using X-PLOR and a model based on the protein component of PDB entry 1ICE, the structure of ICE:Ac-YVAD-CHO complex. The current model was constructed by interactive model-building and refined using X-PLOR. In early stages of model-building, phase refinement using SQUASH significantly improved the quality of electron density maps. The R-factor of the refined model is 19.5% (R_{free}=27.5%) and the stereochemistry is reasonable (r.m.s. deviation of bonds =0.007 Å, angles = 1.31°).

1. J. Rotonda, D. W. Nicholson, K. M. Fazil, M. Gallant, Y. Gareau, M. Labelle, E. P. Peterson, D. M. Rasper, R. Ruel, J. P. Vaillancourt, N. A. Thornberry and J. W. Becker (1996) *Nature Structural Biology*, in press.

MS04.07b.07 STRUCTURE OF A SUPERANTIGEN T-CELL RECEPTOR CHAIN COMPLEX. Roy A. Mariuzza[1], Hongmin Li[1], Emilio L. Malchiodi[1], Xavier Ysern[2], Cynthia V. Stauffacher[3], Patrick M. Schlievert[4], Klaus Karjalainan[5], Barry A. Fields[1], [1]Center for Advanced Research in Biotechnology, University of Maryland Biotechnology Institute, 9600 Gudelsky Drive, Rockville, MD 20850, U.S.A., [2]Center for Drug Evaluation and Research, F.D.A., 5600 Fishers Lane, Rockville, MD 20857, U.S.A., [3]Department of Biological Sciences, Purdue University, West Lafayette, IN 47907, U.S.A., [4]Department of Microbiology, University of Minnesota Medical School, Minneapolis, MN 55455, U.S.A., [5]Basel Institute for Immunology, Grenzacherstrasse 487, Postfach CH-4005, Basel, Switzerland

Superantigens (SAgs) are potent stimulators of the immune system that have been implicated in several major human diseases including rheumatoid arthritis, diabetes mellitus and tuberculosis. SAgs interact with the antigen receptor on T cells as well as molecules of the major histocompatibility complex (MHC) on the surface of antigen presenting cells. These interactions result in the stimulation of an unusually large fraction of the T-cell population. The crystal structure of a SAg that causes food-poisoning, *Staphylococcus aureus* enterotoxin C3 (SEC3), bound to the chain of a T- cell antigen receptor (TCR) has been determined at 3 Å resolution using the high resolution crystal structures of the uncomplexed components as molecular replacement search models. Complementarity determining regions 1 and 2, and, to a lesser extent, hypervariable region 4 of the TCR chain bind in a cleft between the two domains of the SAg. The crystal structure of this complex, along with that of an MHC-SAg complex has enabled the construction of a model for the ternary TCR-SAg-MHC complex. The functional implications of these structures will be discussed.