nicotinamide ring of NADPH. The diphospho bridge and adenosine portion of NADPH was clearly bound to those complexes in typical fashion, but the density for the nicotinamide ring was not visible. That portion of the cofactor was disordered but appeared to extended into solvent, being displaced from its normal binding site by the inhibitor. The width of the binding cleft of *C. albicans* DHFR was significantly larger (1.3-2.0 Å) than the corresponding dimensions of human DHFR and may be a feature that is exploited by the 5-arylthioquinazoline inhibitors. *C. albicans* DHFR crystallized in space group $P2_1$ with two molecules in the asymmetric unit. Final structures had R-factors of 0.155-0.199 at resolution values of 1.6-1.85 Å.

PS04.12.38 STEREOCHEMICAL CONSIDERATIONS IN DRUG DESIGN: THE CRYSTAL STRUCTURES OF HUMAN α-THROMBIN COMPLEXED WITH TWO TRIPEPTIDYL ALDEHYDE INHIBITORS AT 2.1Å RESOLUTION. E. Zhang, R. K. Arni, O. E. Levy#, A. Tulinsky, Department of Chemistry, Michigan State University, East Lansing, MI48824, USA, #Corvas International, Inc. San Diego, CA 92121, USA

Thrombin is a trypsin-like serine protease that plays a central role in thrombosis and hemostasis, inhibitors of which are potentially pharmacologically important as antithrombotics. The structures of many small molecule inhibitors complexed with thrombin reveal that the active site of thrombin has one anionic subsite (S1) and two hydrophobic subsites (S2 and S3) in addition to the catalytic site (Ser-195 and an oxyanion hole) .

The binding of transition-state analog inhibitors, such as peptidyl aldehydes to trypsin-like serine proteases may be modelled by two kinetic steps, a rapid, reversible binding of enzyme and inhibitor involving the three subsites of the active site followed by a slower reversible covalent-like interaction at the catalytic site. Two isomeric peptidyl aldehyde thrombin inhibitors containing 3-guanidylpiperidyl alaninal at the P1 position were designed and synthesized to examine the contributions of steric and dynamic features to selectivity and kinetics. The two inhibitors were identical except for the absolute configuration of the chiral Cγ atom. The inhibitors were soaked into thrombin-hirugen crystals and the structures of the complexes were determined and refined at 2.1Å resolution to R values of ~ 0.15. From the structures it was possible to assign an absolute configuration to each isomer and thus to correlate the structures/configurations with the observed inhibition kinetics. The kinetically fast inhibitor(Ki=5nM) was identified as the R configuration and the more normal slow-tight binding kinetics (Ki=0.3nM) was associated with the S configuration at the P1-Cγ.

The structures of the R- and S- configurations were very similar at the S2 and S3 subsites and the catalytic site, but are different at the S1 subsite. In the R-configuration, the piperidyl ring is in a chair conformation with the guanidyl group forming energetically favorable interactions with Asp-189 of thrombin. In contrast, the piperidyl ring of the S-configuration is in an energetically less favorable boat conformation with its guanidyl interacting with Asp- 189. At the catalytic site, the oxygen atom of the aldehyde group of both isomers is not in the oxyanion hole. These observations suggest that the kinetically slow step is related to the interactions of the boat form in the S1 site.

PS04.12.39 CRYSTAL STRUCTURE OF THE NOVEL CYSTEINE PROTEASE, CATHEPSIN K, IN COMPLEX WITH THE INHIBITOR E-64. Baoguang Zhao, Cheryl A. Janson, Ward W. Smith, Mike McQueney, Christopher Jones, Sherin S. Abdel-Meguid, Departments of Macromolecular Sciences and Protein Biochemistry, SmithKline Beecham Pharmaceuticals, 709 Swedeland Rd, King of Prussia, PA 19406 USA

Cathepsin K is a recently discovered human cysteine protease with significant sequence homology to cathepsin S and cathepsin L. Cathepsin K is abundantly and selectively expressed in osteoclasts, the cells responsible for bone degradation. This observation has led to the suggestion that this enzyme plays an important role in bone resorption. The design of potent, selective inhibitors of cathepsin K should represent a new approach to the prevention of excessive bone loss in diseases such as osteoporosis.

We have determined the three dimensional structure of human cathepsin K in complex with the cysteine protease inhibitor E-64 at 2.2 angstroms resolution. The complex crystallizes in space group $P2_12_12_1$ with unit cell dimensions a = 38.4, b = 50.7, c = 104.9Å. The structure was solved using molecular replacement with the coordinates of papain as the basis for a search model. The resulting electron density confirms that cathepsin K has the identical secondary structure and the same overall fold as papain. The position and conformation of the E-64 inhibitor are clearly evident. We will describe the structure of cathepsin K including the active site of the enzyme and the interactions with the inhibitor and compare this structure with other known cysteine proteases. Knowledge of the structure of cathepsin K will be useful in the structure-based design of inhibitors of the enzyme.

Muscle & Motor Proteins

MS04.13.01 STRUCTURAL BASIS OF MYOSIN MOTILITY. I. Rayment, A.J. Fisher, C.A. Smith, A. Gulick, R. Smith, H.M. Holden and K. Sutoh , Institute for Enzyme Research and Department of Biochemistry, University of Wisconsin, Madison WI 53705, and Department of Pure and Applied Sciences, University of Tokyo, Komba, Tokyo 153 Japan

The mechanism by which chemical energy is transduced into directed movement in muscle and myosin-based motility is a fundamental question in biology. Recently considerable progress has been made towards establishing the molecular basis of the sliding filament model that was proposed over 40 years ago through the determination of the three-dimensional structures of actin (Kabsch et al., 1990, Nature, 347, 37-44) and myosin subfragment-1 (Rayment et al., 1993, Science, 261, 50-58). These have provided a structural framework for a molecular hypothesis for muscle contraction (Rayment et al., 1993, Science, 261, 58-65). Even so many questions remain concerning the structural transitions that underlie the conversion of chemical energy into directed movement. In an effort to understand how ATP hydrolysis is coupled to movement we have determined the structure of a genetically truncated myosin head in the presence of more than seven substrate analogs including MgADP, MgPPi, MgATPγS, MgAMPPNP, MgADP·BeF_x, MgADP·AlF_4, MgADP·VO_4 and several non-ATP derivatives that support tension. These complexes suggest a structural mechanism for ATP hydrolysis and new model for the conformational changes that underlie myosin-based motility.