02.X-1 THE STRUCTURAL CONSEQUENCES OF SITE-DIRECTED MUTATIONS. By R. Bott, M. Ultsch and A. Kossiakoff, Department of Biomolecular Chemistry, Genentech, Inc., South San Francisco, California, USA.

Subtilisin from B. amyloliquefaciens has been used as a model system to protein engineer altered physicochemical characteristics by site-directed mutagenesis. This method has been successful in altering the enzyme's stability, specificity and activity. At several sites that had been identified as being important to enzyme function, the naturally occurring residue was substituted by all nineteen other amino acid types resulting in a wide variety of physicochemical changes. We have chosen a subset of site-directed mutants to study crystallographically aimed at developing relationships between observed structural changes and altered properties.

The structural consequences of site-directed mutations at two sites that affect enzyme activity have been evaluated in detail. The structures of four variants (gln, ala, phe, sulfoxide for met) at position 222 and five variants (leu, ile, asp, lys and asn for gly) at residue 166, as well as several of these variants complexed with peptide inhibitors have been studied at resolution between 2.25-2.0Å. The analyses show that all variants share a pattern of highly localized and subtle structural perturbation (0.3-0.6Å above overall rms deviation). Comparison of variant structures suggests in some cases a systematic pattern of structural alteration that explains the changes in observed kinetic properties. It is hoped that correlations drawn between structural changes and altered properties will lead to the development of improved predictive algorithms to guide rational mutagenesis for design of novel protein properties.

02.X-2 STUDIES OF SPECIFICITY AND CATALYSIS IN TRYPSIN BY STRUCTURAL ANALYSIS OF SITE-DIRECTED MUTANTS. By S. Sprang, T. Standing, R.J. Fletterick, L. Graf, W.J. Rutter and C.S. Craik, Department of Biochemistry, University of California, San Francisco, CA 94143, USA.

We are probing the determinants of catalytic function and substrate specificity in serine proteases by kinetic and crystallographic characterizaton of site directed mutants of rat trypsin. The role of the Aspartyl residue at position 102, common to all members of the serine protease family, has been tested by substitution with Asparagine. At neutral pH, the mutant is four orders of magnitude less active than the naturally occuring enzyme, but its binding affinity for model substrates is virtually undiminished. Crystallographic analysis reveals that Asn 102 donates a hydrogen bond to His 57 forcing it to act as donor to Ser 195. Below pH 6, His 57 becomes statistically disordered. These results suggest that Asp 102 not only provides inductive and orientation effects, but also stabilizes the productive tautomer of Histidine 57.
Three experiments were carried out to alter the substrate specificity of trypsin. Glycine residues at positions 216 and 226 in the substrate binding cavity were replaced by alanine residues in order to differentially affect binding affinities for lysyl and arginyl substrate binding. While the rate of catalysis by the mutant enzymes was reduced in the mutant enzymes, their substrate specificity was enhanced relative to trypsin. The increased specificity was caused by differential effects on k_{cat} towards arginine and lysine substrates. In a third experiment, Asp 189 at the bottom of the specificity pocket was replaced with Lysine in the expectation that specificity of the enzyme might shift to Aspartate. The mutant enzyme is not capable of cleaving at Arg, Lys or Asp, but shows an enhanced chymotrypsin-like specificity.

02.X-3 CORRELATIONS BETWEEN STRUCTURE AND THERMODYNAMIC STABILITY OF PHAGE T4 LYSOZYME. Tom Alber, Jeff Bell, Sean Cook, Julie A. Nye, Sun Daopin, Keith Wilson, Joan Wozniak and Brian W. Matthews, Institute of Molecular Biology, University of Oregon, Eugene, Oregon 97403, USA.

The lysozyme of bacteriophage T4 lysozyme has been used to investigate the structural basis of the thermodynamic stability of proteins. Temperature sensitive (ts) mutants were identified to locate residues that contribute to stability. The ts mutations exclusively alter amino acids with low crystallographic B values and low static solvent accessibility in the wild-type protein. Many types of noncovalent interactions are affected, suggesting that different interactions can make comparable contributions to protein stability. The high resolution X-ray crystal structures of six of the ts mutant proteins provide examples of both localized and dispersed structural changes in response to mutation.

Site-directed mutagenesis has been used to make multiple substitutions at selected sites to determine the relative magnitudes of specific interactions. For example, the thermal stabilities and X-ray crystal structures of lysozymes with 13 different substitutions for Thr 157 and 10 different substitutions for Pro 86 were determined. Observed correlations between native structure and thermal stability suggest that interactions in the folded protein dominate the changes in the free energy of stabilization caused by mutations. One general conclusion from these structural studies of selected and site-directed mutants is that the ability to undergo conformational changes makes proteins surprisingly tolerant of amino acid substitutions.

02.X-4 YEAST tRNA(ASP)-ASPARTYL-tRNA SYNTHETASE COMPLEX AT 7 Å RESOLUTION. By D. Moras, J. Cavarelli, A. Mitschler, A. Podjarny, B. Rees, J.C. Thierry, J.P. Ebel, B. Lorber and R. Giegé, Institut de Biologie Moléculaire et Cellulaire du CNRS, 15, rue René Descartes, 67084 Strasbourg, France.

Dimeric yeast aspartyl-tRNA synthetase and two molecules of its cognate tRNA cocrystallize when using ammonium sulfate as precipitating agent. Two crystal forms are observed: a cubic one (I432) which diffracts to 6 Å resolution at best and an orthorhombic one for which the diffraction patterns extend to better than 4 Å resolution.
The crystal structure of the cubic form has been solved to low resolution using neutron and X-ray diffraction data, without the help of isomorphous derivatives. Two heavy atom-derivatives have now been obtained which confirm the correctness of the solution. Combining MIR phasing, density modification and the use of non crystallographic symmetry leads to a 7 Å resolution model.