PS04.06.13 CRYSTALLIZATON AND REFINED STRUCTURE OF RIBONUCLEASE S COMPLEXED WITH A SUBSTRATE ANALOG. Igor V. Pechik[1] and Gary L. Gilliland[2]. [1]V.A.Engelhardt Institute of Molecular Biology Russian Academy of Sciences, 32 Vavilov St., Moscow, 117984,Russia. [2]Center for Advanced Research in Biotechnology of the University of Maryland Biotechnology Institute and National Institute of Standards and Technology, 9600 Gudelsky Dr., Rockville, MD 20850,USA

A novel procedure was developed to crystallize the complex of RNase S with UpcA, an analog of dinucleotide substrate UpA that has the 5' oxygen substituted with a methylene group. The previous procedure for crystallizing RNase S was based on the use of high concentrations of ammonium sulfate [Wyckoff et al., (1967) J.Biol.Chem., 242, 3749 - 3753]. The modified conditions for crystal growth use only 22-26% saturated ammonium sulfate which is more favourable for UpcA binding, and 50% saturated sodium chloride in 0.1 M sodium acetate buffer at pH 5.0. The concentration of RNase S in droplets was 30 mg/ml with its 1:10 ratio to UpcA [Gilliland et al., (1994) Protein and Peptide Letters, v.1, 60 - 65]. The vapor diffusion procedure appears to be highly stable and reproducable, producing single crystals of the average size about 0.7-1.0 mm. X-ray diffraction data from the unliganded enzyme and its complex with UpcA were collected to 1.8 A resolution with the completeness of 98%.Both structures were refined with final R-factors of 0.167 and 0.17 respectively. Some parts of UpcA molecule can be seen only at the low level of the electron density implying their high mobility. The structure of the complex will be described and compared with the unliganded enzyme. The results of the analysis that focus on structural changes of the protein induced by interactions with the ligand at both the B1 and B2 sites, differences in solvent structure, and the role that water molecules play in the ligand recognition and binding will be presented.

PS04.06.14 CRYSTAL STRUCTURES OF HIGHLY SPECIFIC *ASPERGILLUS* RIBOTOXINS, RESTRICTOCIN AND MITOGILLIN. Xiaojing Yang[1], Sergio Martinez[2], Janet Smith[2] and Keith Moffat[1], [1]Department of Biochemistry and Molecular Biology, The University of Chicago, Chicago, IL 60637, USA and [2]Department of Biological Sciences, Purdue University, West Lafayatte, IN 47907, USA

Two crystal structures in the *Aspergillus* ribotoxin family, restrictocin and mitogillin, are reported. The *Aspergillus* ribotoxins are a group of ribonucleases that specifically cleave a single phosphodiester bond in a highly conserved region of eukaryotic 28S ribosomal RNAs and thereby inhibit protein synthesis. The crystal structure of restrictocin is determined by single isomorphous replacement and anomalous scattering techniques and refined to 1.7Å resolution using synchrotron Laue diffraction data. The crystal structure of mitogillin is determined by the difference Fourier method benefited by the high isomorphism between restrictocin and mitogillin crystals. The overall structures of restrictocin and mitogillin are identical except one residue where Ser25 of restrictocin is replaced by Asp25 of mitogillin. The crystal structure reveals a structural core in which a 5-stranded β-sheet is packed against a 3-turn α-helix, which can be well aligned with that of ribonuclease T1. Large peripheral loops near the active site construct a concave surface for substrate RNA binding. A lysine-rich loop is suggested to be responsible for the high substrate specificity by a docking model derived from the restrictocin structure and an NMR structure of a 29-mer RNA substrate analog. A large loop domain is also indicated to be involved in the cell entry activity of the *Aspergillus* ribotoxins.

PS04.06.15 CRYSTAL STRUCTURE OF TRYPTOPHANYL-TRNA SYNTHETASE COMPLEXED TO INDOLMYCIN, A SPECIES-SPECIFIC INHIBITOR Yuhui Yin and Charles W. Carter, Jr., Department of Biochemistry and Biophysics, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7260 USA

Indolmycin is a tryptophan analog containing indole joined to an oxazolone ring. It selectively inhibits prokaryotic Tryptophanyl-tRNA synthetases (TrpRS) with an affinity (K_i = $9x10^{-6}$ M) at least 10^3 fold greater than that for eukaryotic TrpRS and is thus a potential candidate for antibacterial drug design. We have solved the crystal structure of an indolmycin:ATP:TrpRS complex to provide a structural basis for increasing its binding affinity while preserving the species specificity. Tetragonal crystals of this complex were prepared using a high excess (10 mM) of ATP. The structure, solved at 3.0 Å resolution by molecular replacement, has several unexpected features. TrpRS contains two domains: a Rossmann fold containing the active site, and a helical domain. The indole moiety binds to the active site in a manner similar to that observed previously in the Trp-5'AMP complex, involving a hydrogen bond to Asp 132. A new hydrogen bond is formed between Asp 132 and the nearby His 43. The pH-dependence of this hydrogen bond may help explain a 13-fold increase in Vmax/Km observed at high pH. The oxazolone ring makes hydrogen bonds both to His 43 and to Gln 147 on the opposite side of the active site. The ATP molecule adopts an unusual orientation, with the adenine ring facing the active site opening and the triphosphate in the space occupied by the adenine moiety of the Trp-5'AMP adenylate intermediate (Doublié, et al., *Structure*, 3:17-31, 1995). It makes contacts with both of the "signature" sequences characteristic of class I aminoacyl-tRNA synthetases. The triphosphate shifts the KMSKS loop from its configuration in the adenylate complex, such that Lys 192 makes contact with the beta phosphate. The KMSKS loop rearrangement is correlated with an 8° rotation of the helical domain, relative to the Rossmann fold domain. This conformation resembles that of a ligand-free structure obtained by dissociation of the product, Trp-2',3'-ATP, from tetragonal crystals. The tertiary structure of the indolmycin:ATP:TrpRS complex therefore differs from that of the Trp-5'AMP intermediate complex. Movement of the helical domain relative to its position in that structure suggests that formation of the adenylate triggers a conformational change which might be important for tRNA recognition.

(This work supported by NIH GM48519-02)