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 favorable 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 I:1 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 reproducible, 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.

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 1.7A 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 beta-sheet is packed against a 3-turn alpha-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.