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Thermodynamic Studies of Ligand-binding and Conformational Changes of *B. stearothermophilus* Tryptophanyl-tRNA Synthetase (Trp-RS). Violetta A. Weinreb, Charlie W. Carter, Jr., Dept. of Biochemistry & Biophysics, Univ. of North Carolina at Chapel Hill, CB 7260, Chapel Hill, NC 27599 USA.

TrpRS catalysis involves ligand-dependent conformation changes. Binding and chemical transformation of ATP appear to be key to these changes. To uncouple binding from conformational free energies and determine the influence of catalytic residues, we are examining fluorescence (1) and CD changes induced by ATP binding as well as chemical and thermal denaturation, compared to previous isothermal titration calorimetry experiments. Details on the data collection and processing of the data will be included in presentation. Equilibrium constants and free energy changes have been determined for fluorescence changes induced by ATP ($K_{eq} = 403 \mu\text{M}$) and chemical denaturation ($K_D = 164$). We hope to determine the origin of fluorescence quenching by comparing fluorescence and CD with structures, and to use stabilization by bound ligands to determine binding affinities (2). Similar studies involving KMSKS mutants should reveal important aspects of that loop's function. (Supported by NIGMS 48519)

1. Edgell MH, Sims DA, Pielak GJ, Yi F. *Biochemistry* 2003;42(24):7587-7593.
2. Weber PC, Wendoloski JJ, Pantoliano MW, Salemme FR. *J. Am. Chem. Soc* 1992;114:3197-3200