PS04.10.29 PRELIMINARY DIFFRACTION STUDIES OF THE REGULATED FORM OF CHICKEN SRC PROTEIN TYROSINE KINASE. J. C. Williams, A. Weijland, S. A. Courtneidge[1], G. Superti-Furga, and R. K. Wierenga, EMBL, Postfach 102209, D69012 Heidelberg, Germany, [1]SUGEN, Redwood City, CA 94063 USA

We present here preliminary diffraction data of the regulated form of the chicken Src Protein Tyrosine Kinase. The crystals diffract beyond 2.7Å with low mosaicity (<0.2°) at room temperature using syncrotron radiation at beam lines BL4 and BL19 at the ESRF, Grenoble. Due to severe radiation damage, the data was collected under cryo-conditions with a substantial increase in mosaic spread (1.1°). The native data set is 88% complete to 2.7Å (75%-last shell) and a mercury data set is 75% complete to 3.0Å (75%-last shell). The crystals are orthorhombic ($P2_12_12_1$) with cell constants of a=54, b=89, c=99 with one molecule per assymetric unit and a solvent content of 53%. The Rdiff between the native and the mecury data sets is 33%.

Molecular Replacement calculations have been initiated, but prove to be difficult. Cocrystallization and soaking experiments with additional heavy atom compounds as well as seleno-deriviates are underway.

PS04.10.30 STRUCTURES OF ELONGATION FACTOR TU-GDP COMPLEXED TO ANTIBIOTICS: GE2270 AND TETRACYCLINE. Kenton Abel, Suet Mui, Marilyn Yoder and Frances Jurnak, Department of Biochemistry, University of California, Riverside, California 92521

The three-dimensional structures of two EF-Tu-GDP:antibiotic complexes have been solved by X-ray diffraction analyses. The EF-Tu-specific antibiotic, GE2270, crystallizes as a complex with EF-Tu-GDP in space group $P2_1$, with two complex molecules in the asymmetric unit. The structure has been solved by molecular replacement and refined to a final crystallographic R factor of 18.6% at Å resolution of 2.5 Å. In contrast to EF-Tu-GDP models which have been previously reported, the Effector I region (44-58) has not been removed by protease and is well-ordered in the present structure. The Effector I loop in the guanine nucleotide-binding domain forms an antiparallel beta ribbon, the tip of which extends to the third domain. A comparison of the Effector I loop with the analogous loop in the Thermus thermophilus EF-Tu-GTP structure reveals that 13 amino acids undergo a dramatic conformational change from beta to alpha structure. The antibiotic could not be assigned unambiguously in the residual electron density.

The structure of a trypsin-modified EF-Tu-GDP-tetracycline complex has been solved and refined to a crystallographic R factor of 20.9% at Å resolution of 2.7 Å. The current model consists of six copies of EF-Tu amino acids, 9 through 42 and 59 through 393, six copies of MgGDP and six tetracycline molecules. The phenolidiketone moiety of tetracycline coordinates to the Mg^{2+} and forms hydrogen bonds to the semi-invariant Thr25 and the invariant Asp80. The binding motif suggests that tetracycline may interact with other guanine-nucleotide binding proteins. The finding that tetracycline binds to the active site region of EF-Tu suggests that the accepted mode of inhibition of protein synthesis, via tetracycline binding to the A site of the ribosome, needs to be re-evaluated. The present results also indicate possible modes of EF-Tu binding to the EF-Tu-specific antibiotic, kirromycin, as well as to the chemotherapeutic agents, daunorubicin and taxol.

PS04.10.31 THREE-DIMENSIONAL STRUCTURE OF THE *E. COLI* ELONGATION FACTOR TU-TS COMPLEX AT Å RESOLUTION OF 2.3 Å. Susan Heffron, Jacqueline Vitali, Brian Schick, Kenton Abel and Frances Jurnak, Department of Biochemistry, University of California, Riverside, California 92521

The Escherichia coli EF-Tu-Ts complex crystallizes in space group $P2_12_12_1$ with cell parameters of a=81.1 Å, b=109.9 Å and c=207.5 Å. The latter crystals diffract to a resolution of 5 Å, but prior to data collection, are converted into a form which diffracts to 2.2 Å or better by the gradual removal of solvent. With two copies of the EF-Tu-Ts complex (75 KDa each) in the asymmetric unit, the phase solution represents a challenge. None of the Pattersons for five heavy atom derivatives have Harker peaks that are significantly above background. As a consequence of conformational flexibility, molecular replacement calculations do not reveal any plausible solutions using a highly refined EF-Tu structure, representing 30% of the asymmetric unit, as a search model. Ultimately, the EF-Tu-Ts structure has been solved by a creative combination of MIR and molecular replacement methods, using fragmented search models representing 12% of the asymmetric unit. The details will be presented in the poster. The structure is now refined to a resolution of 2.3 Å and a crystallographic R factor of 23% in the absence of solvent. The structure reveals not only the atomic details of the EF-Ts binding site on EF-Tu, but the striking structural similarity between EF-Ts and other ribosomal proteins.

PS04.11.29 SUGAR BINDING IN CRYSTALLINE CYCLODEXTRIN GLUCANOTRANSFERASE. K. Harata[1], N. Ishii[1], K. Haga[2], M. Aoyagi[2], K. Yamane[2], [1]National Institute of Bioscience and Human-Technology, 1-1 Higashi, Tsukuba, Ibaraki 305, Japan, and [2]Institute of Biological Sciences, University of Tsukuba, Tsukuba, Ibaraki 305, Japan

Cyclodextrin glucanotransferase (CGTase) is an enzyme which produces cyclodextrins by the degradation of starch. The enzyme from alkalophilic Bacillus sp.1011 is stable in markedly wide pH range. To elucidate the mechanism of enzyme action, the crystal structure of the acarbose complex has been investigated by the Xray method. The acarbose molecule is bound in the active center and has short contacts with Asp229, Glu257, and Asp 328 which have been considered as catalytic residues. Asp229 and Glu257 are located at the secondary hydroxyl side of acarbose in the distance of hydrogen-bonding contact with a glycosidic oxygen atom. On the other hand, Asp328 is hydrogen-bonded to O5 of the adjacent pyranose ring. The result is consistent with the proposed model of the catalytic reaction. The crystal contains two independent CGTase molecules. In both molecules, acarbose is bound in the same manner, but differences are observed in contacts with individual amino acid residues.

Crystal of the complex was obtained by the co-crystallization in the presence of 1mM acarbose in the same condition as used in the crystallization of isomorphous native crystal. The structure was refined to the current R-value of 0.17 for the 2.2Å resolution data. The structural study of acarbose complexes of CGTase mutants are in progress.