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Cysteine plays a major role in growth and survival of the human protozoan parasite *Entamoeba histolytica.* In bacteria and plants, cysteine is synthesized by a two step cysteine biosynthetic pathway catalyzed by serine acetyltransferase (SAT) and O-acetylserine sulfhydrylase (OASS) in first and second step respectively. These two enzymes interact to form a decameric cysteine synthase complex. The pathway is regulated by two steps; association and dissociation of cysteine synthase complex and feedback inhibition of SAT by cysteine but this mechanism is absent in *E. histolytica* [1]. The OASS structure from *E. histolytica* revealed a C-terminal end helix which occupies its own groove near the catalytical cavity and it was proposed earlier this may be the reason for loss of interactions between OASS and SAT [2]. We present here the crystal structure of serine acetyltransferase isoform1 from *E. histolytica* (EhSAT1) at 1.77 Å, in complex with its substrate serine at 1.59 Å and inhibitor cys at 1.78 Å resolutions. All the three forms crystallized in R3 space group and contained one molecule per asymmetric unit. This is the first crystal structure of a trimeric SAT, unlike hexameric SAT structures reported in *E.coli* and *H. influenzae*. The difference in oligomeric state is due to the differently oriented N-terminal region of the EhSAT1, which has very low sequence similarity to known structures and also differs in charge distribution at trimeric interface. The Ser and Cys bind to the same site in EhSAT1 which confirms that Cys is a competitive inhibitor of Ser. The disordered C-terminal region and the loop near the active site are responsible for solvent accessible acetyl Coenzyme-A binding site and thus lose inhibition to acetyl Co-A by the feedback inhibitor Cys. Docking and fluorescence studies show that EhSAT1 C-terminal mimicking peptides can bind to o-acetyl serine sulfhydrylase (EhOASS), while native C-terminal peptide does not show any binding. To test further, C-terminal end of EhSAT1 was mutated and found that it inhibits EhOASS, confirming modified EhSAT1 can bind to EhOASS. Thus it is clear that *E. histolytica* had modified its C-terminal to escape the complex regulatory mechanism just by replacing two residues [3].

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Protein structure determination using Saturn A200 CCD at SPring-8 by MAD phasing

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X-ray area detectors consisting of CCD array are now indispensable to synchrotron beamlines for macromolecular crystallography owing to the high throughput and low noise. The CCD detector enables rapid collection of high resolution data at the synchrotron and also in the high-brilliance home laboratory.

Saturn A200 is the latest 2x2 CCD array detector of Rigaku and employs an array of four CCD chips providing a 203mm x 203mm active area. We examined the performance of the Saturn A200 at the BL26B1 beamline at the SPring-8 by structure determination of an enzyme BxlA, beta-xylosidase from *Streptomyces thermoviolaceus*. A three-wavelength MAD dataset of a SeMet crystal of BxlA and a single-wavelength dataset of a native BxlA crystal were collected and processed. After automated procedure of CCP4 suite, 14 of 20 seleno-methionine sites were found in an asymmetric unit and resulting electron density map was auto-traceable.

We realized that the Saturn A200 has sufficient perfomance for MAD phasing and structure refinement. We will study further and continue to improve the detector.

Keywords: CCD detector, protein crystallography application, MAD phasing

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PSI-Biology partnership projects at the joint center for structural genomics

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The Joint Center for Structural Genomics (JCSG) is one of four Centers for High-Throughput Structure Determination (CHTSD) funded as part of the NIH/NIGMS PSI-Biology program. Ten Consortia for High-Throughput-Enabled Structural Biology Partnerships (SBPs) are also funded as part of the PSI-Biology Network. The SBPs are focused on challenging and high-impact biological topics and have each proposed a set of biologically relevant targets for structure determination by the CHTSDs.

The JCSG is partnered with two SBPs. In collaboration with Dr. R. Fletterick and colleagues, we are exploring the regulatory transcription factor machinery essential to embryonic stem cells and induced pluripotent stem cells and, with Dr. J. Williamson and Dr. D. Salomon, we are investigating the role of ribonucleoprotein complexes that regulate changes in gene expression accompanying T-cell activation. Both of these partnership projects involve large multi-domain human proteins, protein-protein complexes and protein-nucleic acid complexes. We have also developed a strategy to identify and structurally characterize novel domains with these target proteins based on extensive bioinformatic analyses of the prioritized target lists. Both bacterial and mammalian protein production are being pursued for structure determination. In parallel, our partners are identifying and characterizing stable protein and protein/nucleic acid complexes, which represent promising structural targets for the JCSG pipeline. As new structures are solved, we work closely with our partners to design follow-up biological experiments. We will give an overview of these two projects outlining our strategy and highlighting the progress to date, including the first structures to emerge from these new collaborations.

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Keywords: high throughput structural biology, DNA RNA binding protein, stem cells