

Abstract No. Brya0069

**Se-Met Structure Determination of Lysine-2,3-Aminomutase from *C. Subterminale* SB4**

Bryan W. Lepore (Brandeis Univ.), Frank Ruzicka (Univ. of Madison, WI)

Beamline(s): X25

**Introduction:** Lysine-2,3-aminomutase is a 280,000 kDa hexameric enzyme which converts L-lysine to beta-lysine by initiating a substrate radical using S-adenosyl methionine and a [4Fe-4S] cluster, then stabilizing that radical with pyridoxal-5'-phosphate.

The rearrangement reaction is analogous to those performed by vitamin B12, and is novel to the reactions catalyzed by pyridoxal-5'-phosphate. When *Clostridia* are grown on L-lysine as the sole carbon source, this enzyme catalyzes the first step in the degradation of lysine to ammonia and acetyl-CoA, which satisfies the organisms' need for metabolizable carbon skeletons and energy. In addition, beta-lysine can be found as a substituent on antibiotics from various microorganisms. The only known natural way to form beta-lysine is by the action of lysine-2,3-aminomutase on L-lysine.

This enzyme has little similarity to known sequences, and threading experiments cannot clearly distinguish the enzyme's fold. Phasing off of the intrinsic iron-sulfur cluster has not worked, most possibly due to the low (3.5 Angstroms) resolution available, so alternative phasing methods were sought, including use of Se-Methionine. While a successful growth protocol was worked out which yielded crystals with ~80% Selenium incorporation, improved crystal growth conditions including useage of Sodium Malonate were found, and Se-Met crystals grown this way were frozen readily.

**Methods and Materials:** Se-Met crystals were grown in Na-EPPS pH8.0, Na-Malonate, PEG 8K and PEG 200, and frozen in PEG 8K and PEG 200. The crystals belong to spacegroup C2, with reproducible parameters. Selenium edge and peak data were collected at beamline X25 of the NSLS from one crystal. This was the most data that was possible to collect due to time constraints, which was generously granted to me by my lab members who were working on other projects.

**Results:** The data sets are nearly 90% complete to 2.3 Angstroms, and the crystals were robust in the beam. There are either 30 or 40 Seleniums per asymmetric unit, which almost necessitates useage of automated Patterson superposition programs or direct methods. The best results so far have been obtained with the program SOLVE/RESOLVE (Terwilliger, et. al.), which seems to be able to discriminate a solvent/protein boundary, however there are too many incorrect heavy atom sites that we cannot obtain clear image of the protein at resolutions between 2.3 and 10 Angstrom. A very troubling aspect of this data set is the gross inconsistency of EXAFS scans at the end of the edge wavelength and beginning of the peak, compared to the EXAFS taken prior to the edge wavelength (the first wavelength collected), an aspect that was unresolved by the beam technician on duty. Secondly, the crystal precessed out of the beam at the end of collection, which prevented the maximization of data completeness.

**Conclusions:** Our data set is good, but not good enough to clearly distinguish enough correct heavy atom sites. Our plan is to grow more of the same crystals, and collect a larger data set including a high-energy remote wavelength, which will improve the chance of locating sites. Improving the freezing conditions will help our R-merge in the high resolution bins also.

**Acknowledgments:** I thank my lab members for offering me time at the end of their 1-day run and Mike Becker for expediting my safety approval form so that I could bring the freshly formed new crystals that had never seen beam before down to X-25 for an experiment.

**References:** Terwilliger, T.C. and J. Berendzen. (1999) "Automated structure solution for MIR and MAD". *Acta Crystallographica* D55, 849-861.