X-ray Crystallographic Study of *Helicobacter pylori* Aminodeoxychorismate Lyase and Shikimate Kinase (HpSK) Inhibitors

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TOPIC I. X-ray crystallographic study of structure-function evolution in the aminodeoxychorismate lyase

Helicobacter pylori is a gastric pathogen that induces chronic inflammation and may habit in this unique ecological niche for a life-time period, progressing into severe peptic ulcers or gastric carcinoma. The de novo synthesis of folate that requires several enzymes is unique to microbial cells and parasites but absent in animals. Aminodeoxychorismate lyase is one of the important enzymes in folate pathway to fully explore folate biosynthesis with emerging strategies in drug discovery. For solving the crystal structure, we need to use different wavelengths to obtain the MAD (multiple-wavelength anomalous dispersion) data, and the powerful synchrotron source will help us to obtain high resolution data that we can get more details of our structure.

In this study, several protein crystals were tried to be collected by using synchrotron radiation source in the Spring-8 beamline 12B2 and NSRRC 13B1. We tried to use seleno-methionine (Se-Met) crystals to get the structural phase. We had scanned the selenium absorption edge to determine which energy we can collect. Several protein crystals had been tried to collect data, and the best one crystal can diffract to about the resolution of 2.4Å. Besides, we also got the native crystal diffraction data set about the resolution of 2.0Å. As follows, we only show the best data set here.

TOPIC II. Structure-based discovery of *Helicobacter pylori* shikimate kinase (HpSK) inhibitors

Shikimate pathway plays an important role in aromatic amino acid biosynthesis in bacteria, fungi, and plants, but not mammals, in which enzymes of this pathway represent attractive targets for the development of new antimicrobial agents, herbicides and antiparasitic agents. We have determined the structure of the fifth enzyme, shikimate kinase (HpSK) that catalyzes the specific phosphorylation of the 3-hydroxyl group of shikimic acid in the presence of ATP in its apo form (1.8 Å) and the HpSK·shikimate·PO_4 (2.3 Å) binary complex structures. The binary structure reveals induced fit movement on substrate binding and the detailed enzyme-substrate interactions. Several highly conserved charged residues including Asp33 (in a conserved Asp31Thr/Ser32Asp33 motif), Arg57, and Arg132 (interacting with shikimate) are identified, guiding the development of novel inhibitors of shikimate kinase. We have conducted virtual drug screening utilizing the GEMDOCK molecular docking tool to analysis the 65535 compounds of Maybridge database from the ZINC database. Several compounds were identified that inhibited the HpSK activity with IC50 values. These included compound 8 (IC50 = 24 μM) and others with the micromolar range. In this study, we tried to collect the complex structure of HpSK and inhibitors. This study may be useful in the development of potent and more specific inhibitors of the HpSK to inhibit the growth of *H. pylori*.

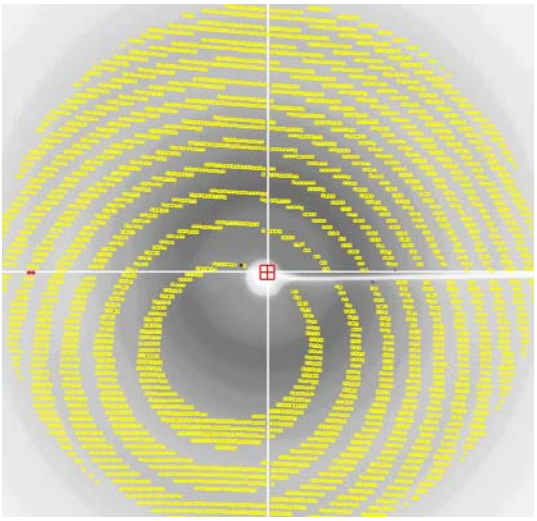

Figure 3. Index the 90° diffraction pattern of aminodeoxychorismate lyase Se-Met crystal in HKL2000