Structural Research in *Helicobacter pylori* Targets for Discovery of Anti-bacterial Agents and in Biocatalysts of Chiral Reaction

Helicobacter pylori is a gastric pathogen that infects approximately half of the human population. Persistent colonization is associated with chronic inflammation processes, gastric atrophy, gastric adenocarcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma. In 1994, the World Health Organization's International Agency for Research on Cancer has declared H. pylori as a group I carcinogen. Our laboratory has been interested in the molecular pathogenesis of H. pylori, focusing on several virulence factors including vacuolating toxin (VacA), cytotoxin associated antigen (CagA), and the blood group antigen-binding adhesion (BabA2) related to more severe clinical outcomes. Within a 10-year period of research, these factors have been well characterized among Taiwanese clinical isolates. Together with worldwide results, H. pylori is recognized as a species of high genetic heterogeneity with respect to virulence determinants, hence markedly variation in virulence and disease risk. Additionally, we found that VacA entered into epithelial cells via a newly defined endocytic pathway and that two types of VacA presented distinct cell-type specificity due to various cell-surface receptors. We also found that the failure of the efficacy of H. pylori lansoprazole-based triple therapies comes mainly from the primary resistance to antibiotics of clinical isolates in Taiwan and that resistant H. pylori strains are associated with antibiotic resistance and superior internalization activity, protecting them against antibiotic treatment. These results together encourage the search for new antimicrobial agents or effective combinations of existing drugs. We have currently targeted enzymes in the shikimate pathway in an aim to discover potent inhibitors based on a structure-based approach. The second focus of the structural project is important biocatalysts in the chiral reactions since chiral agents are a rapidly growing segment in the pharmacological and biotechnological market. Indeed, we have solved several members of the nitrilase superfamily that are of great interest in synthetic industries where they are used for mass production of acidic products. By use of the combined structural/molecular biological approach, we aim to engineer biocatalysts with desired properties.

Topic I: Structure-based discovery of potent inhibitors against enzymes in the shikimate pathway

Given the need of new antibacterial therapy to overcome the problem of antibiotic resistance and the emergence of new infections diseases, we have been interested to investigate proteins in bacterial growth or pathogenesis. To this end, we have chosen the shikimate pathway due to its role in aromatic amino acid biosynthesis in bacteria, fungi, and plants, but not mammals. Thus far, two structures of five target enzymes have been determined: (1) The first structure is 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) form; and (2) The structure of 3-dehydroquinate synthase that catalyzes the conversion of 3-deoxy-D-arabino-heptulosonate 7-phosphate (DAHP) to 3-dehydroquinate (DHQ) is also recently solved. Based on the determined HpSK structures, we have performed virtual docking and scoring utilizing the

Beamlines

13B1 Protein Crystallography
13C1 Protein Crystallography
SP12B2 Protein X-ray Crystallography

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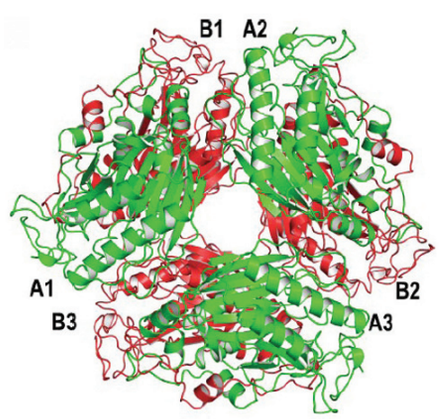
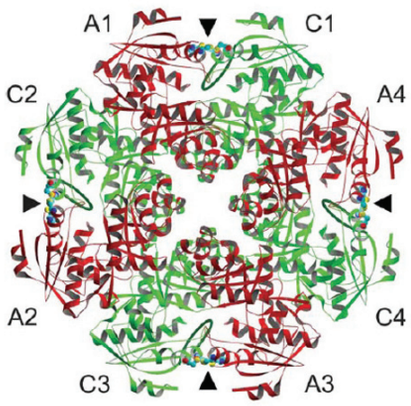
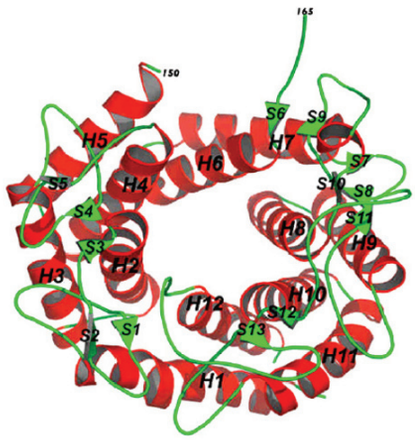


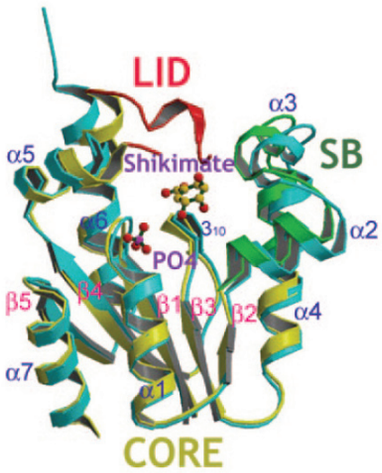
Formamidase (AmiF)



N-Acylamino acid Racemase (NAAAR)



N-Acetyl-D-glucosamine 2-Epimerase
(GlcNAc 2-epimerase)



Shikimate Kinase (HpSK)

Fig. 1: (a) O *K*-edge XANES spectra of all ZnO QDs and powder. Inset displays magnified O *K*-edge features following background subtraction; (b) Resonant Raman scattering spectra of all ZnO QDs and bulk ZnO.-test

GEMDOCK molecular docking tool to analyze compounds from Maybridge database and those from the ZINC database (a free database from University of California, San Francisco). Of 100 top-ranking compounds, five were identified that display inhibitory effects against the HpSK activity. The best one had an IC_{50} value of 24 μM. The three-dimensional quantitative structure activity relationship (3D-QSAR) will be employed to quantitatively predict pharmacophore properties to seek for potent specific HpSK inhibitors.

Topic II: Structure-function studies of biocatalysts in the chiral reactions

We have been interested in the structure-function studies of several biocatalysts involved in chiral reactions. The first determined structure is *N*-carbamoyl-D-amino-acid amidohydrolase (D-NCAase) that hydrolyzes *N*-carbamoyl D-amino acids to produce valuable D-amino acids using the methods of multiple isomorphous replacement with anomalous scattering. We have further determined the crystal structures of inactive mutants, C172A or C172S in its free and liganded forms. These structures clearly reveal a characteristic fold of the nitrilase superfamily and a C172-E47-K127 triad. The active cysteine is postulated to attack a carbon in specific nitrile- or amide-hydrolysis or amide-condensation reactions, resulting in synthesis of various natural products. To our knowledge, this is the first structure of the nitrilase superfamily that has a substrate in the active site for the clear delineation of Glu-Lys-Cys catalytic triad at the atomic resolution. Based on a rational design, enzymes are thus modified to improve oxidative resistance and thermostability. We have recently determined another member of the nitrilase superfamily, formamidase (AmiF) from *H. pylori*

that plays a major role in producing ammonia to protect against gastric acidity or as a nitrogen source or a cytotoxic molecule, enabling *H. pylori* to adapt into such an exclusive environment. The liganded inactive mutant structure was also determined, presenting a small formamide-binding pocket and hence restricted substrate specificity.

Deinococcus radiodurans N-acylamino acid racemase (NAAAR) that catalyzes racemization of N-acylamino acids can be used in concert with an aminoacylase to produce enantiopure α-amino acids, a process that has potential industrial applications. We have determined the 1.3-Å NAAAR structure, demonstrating a homooctamer in which each subunit has an architecture characteristic of enolases with a capping domain and a $(\beta/\alpha)_7$ barrel domain. The liganded Mg^{2+} and *N*-acetyl-L-glutamine·Mg^{2+} NAAAR structures reveal the Lys170-Asp195-Glu220-Asp245-Lys269 catalytic framework and four subsites (catalytic site, metal-binding site, side-chain-binding region, and a flexible lid region) in the binding pocket. Of different enolases, there is high conservation in catalytic and metal-binding sites while less in side-chain-binding region and a flexible lid region in substrate recognition, suggesting a divergent evolution that leads to functionally distinct enzymes.

N-Acetyl-D-glucosamine 2-epimerase (GlcNAc 2-epimerase) catalyzes the reversible epimerization between N-acetyl-D-glucosamine (GlcNAc) and Nacetyl-D-mannosamine (ManNAc). In couple with N-acetyl-D-neuraminic acid lyase, GlcNAc and pyruvate can then be reacted to produce sialic acid for biological and pharmacological applications. We have recently determined the apo-form structure that demonstrates an (α / α)6 barrel fold, homologous to porcine GlcNAc 2-epimerase as well as a number of glycoside hydrolase enzymes and other sugar-metabolizing enzymes. One side of the barrel structure consists of short loops involved in dimer interactions. The other side of the barrel structure is comprised of long loops containing six short beta-sheets, which enclose a putative central active-site pocket. Based on the structure and kinetic analysis, H239 and H372 are proposed to serve as the key active site acid/base catalysts. These results suggest that the (alpha/ alpha)6 barrel represents a steady fold for presenting active-site residues in a cleft at the N-terminal ends of the inner alpha helices, thus forming a fine-tuned catalytic site in GlcNAc 2-epimerase. ■

Experimental Stations

Protein Crystallography
Protein X-ray Crystallography

Publications

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