Enzymes and their applications

Enzymes are catalysts that are vital for living cells to maintain their cellular activities. The rates of reactions are increased tremendously by enzyme catalysis. The power of enzyme catalysis is usually manifested through nature of the active site where particular residues or cofactors react with substrates, leading to a series of chemical reactions which require much less activation energy when compared non-catalyzed reactions. The specificity of enzyme active sites and cofactors also enable enzymatic reactions to be regio- and stereo-specific. Through evolution, nature find means to control fidelity and accuracy of reactions so that unwanted compounds which may be toxic for cells are minimized.

Taking advantage of molecular nature of enzymatic reactions, various enzymatic reactions have been applied in industries, playing vital roles in the production of foods, chemicals and pharmaceuticals. With the increasing demand for cost- and energy-efficient production due to limited world resources, chemicals and pharmaceuticals industries have been using more enzymatic reactions in manufacturing so as to save energy and reduce toxic waste. In-depth knowledge of enzyme mechanisms is needed so that the needed enzymes can be engineered or tailored to achieve the desired biocatalytic behavior.
Enzymes are also important as drug targets for battling pathogenic microbes or metabolic disorders. Understanding reaction mechanisms and kinetics of enzymes from various species and knowledge of the differences among specific variants are vital for finding specific inhibitors against a particular target. Such inhibitors may eventually lead to discovery of new drugs.

Research in Chaiyen laboratory of Enzyme Catalysis and Engineering

The main goal of our research is to gain an in-depth understanding of the reaction mechanisms of selected redox and aldolase enzymes. A thorough understanding of enzyme catalysis will allow us to engineer enzymes for applications in biocatalytic process. The knowledge about enzyme mechanisms and kinetics is also useful for devising enzymes and their reactions to be used as bioreporters and for discovering new drugs against diseases.

Enzymology is a multidisciplinary field spanning biochemistry, physical chemistry and organic chemistry. We employ a wide range of physical and biochemical tools and techniques for studying enzymatic reactions. We also augment our laboratory’s research and techniques capabilities through collaborations with leading Thai and international universities.

1. Kinetics. Kinetics, especially transient kinetics, allows us to measure rate constants associated with individual steps of reactions. We have two stopped-flow spectrometers and one rapid-quench instrument which can detect any spectroscopic changes ~ 0.002 s after substrate and enzyme mixing. We are also well equipped with anaerobic facilities i.e. glovebox and anaerobic train for studying redox reactions or preparing regents which are sensitive to air.

2. Spectroscopy. Because the enzymes being investigated contain flavin, PLP, or metal. we routinely use absorbance/ fluorescence/luminescence spectroscopy to detect changes associated with ligand binding and enzymatic process. Through collaborations, EPR spectroscopy is used to probe environments of metal-bound in metalloenzymes.

3. Kinetic isotope effects. Primary kinetic isotope effects and solvent kinetic isotope effects are useful mechanistic probes to identify which bond breakage or formation is the rate-determining factor for the chemical step observed in kinetic experiments. We often use kinetic isotope effects to assess whether the transfer of group or proton of interest is a major factor controlling catalysis.

4. Functions of active site residues. We use site-directed mutagenesis approach to mutate the residues speculated to be important for the catalysis. When data of mutants and wild-type
enzymes are compared, full understanding of enzyme structure and function can be developed.

5. Structures. Enzyme structures are studied with X-ray crystallography or NMR spectroscopy. We have been collaborating with crystallographers and NMR experts to study three-dimensional structures of our enzymes.

6. Computational Chemistry. Insightful analysis of enzymatic reactions using quantum mechanics and theoretical calculations are useful for postulating critical physical parameters that govern catalysis. Information from theoretical studies is also compared to experimental data obtained from kinetics or thermodynamics experiments.

**Dr. Pimchai Chaiyen’s Laboratory : Current Research**

Currently, we are investigating more than 10 enzymatic reactions. Most of the reactions can be classified into 4 groups according to enzyme applications.

1. Enzymes that are useful for biocatalysis : Flavin-dependent enzymes such as p-hydroxyphenylacetate hydroxylase (HPAH) can perform regio-specific hydroxylation of phenolic compounds. We are currently exploring HPAH potential in synthesizing catecholic drugs or anti-oxidants.

Pyranose 2-oxidase (P2O) can catalyze regio-specific oxidation at C2-position of pyranoses. P2O reaction is useful for synthesis of sweetener and rare sugars. A few more enzymatic systems are being developed for potential use in pharmaceutical industry.

2. Enzymes that are useful for biorefinery process : In order to support sustainable development of Thailand, we are currently exploring a few enzymatic systems that can convert waste or by-products from biomass to useful compounds such as bioplastics or biogas.

3. Enzymes that are useful as bioreporters : Bacterial luciferase is a flavin-dependent enzyme that catalyzes light-emitting reactions. We are currently developing gene reporter systems to be used in bacteria and yeast.

4. Enzymes that can be potential drug-targets : The enzyme serine hydroxymethyl transferase (SHMT) is an indispensible anti-malarial target. We are studying its reaction mechanisms so that any difference between host and parasite enzymes can be delineated and used for
designing specific inhibitors against malaria. The human SHMT is also a good target for cancer therapy.

Current research projects of HPAH
1) Reaction of C2 with substrate analogues and applications of C2 as a biocatalyst
   Investigator:
   Taweesak Dhammaraj
2) Control of oxygen reactivity in C2
   Investigator:
   Pirom Chenprakhon
   Duangthip Trisrivirat
3) Biological significance of C1 and C2
   Investigators:
   Dr. Kaseka Kasevayuth
   Dr. Kittisak Thotsaporn
4) Novel oxygenases
   Investigators:
   Panu Pimviriyakul
   Kittisak Thotsaporn
   Litavadee Chuaboon