Division of Biochemistry
- Chemistry of Molecular Biocatalysts -

http://biofun.kuicr.kyoto-u.ac.jp/index-e.html

Prof
HIRATAKE, Jun
(D Agr)

Assist Prof
WATANABE, Bunta
(D Agr)

Students

IKEUCHI, Hideyuki (D1)
ASAI, Tomoki (M2)
IKEGUCHI, Keiji (M1)
OTOKAWA, Takuya (M1)
TABUCHI, Yukiko (M1)
YAMAMOTO, Yuki (M1)

Scope of Research

Our research covers the comprehensive understanding of the physiological roles of biocatalysts (enzymes), as well as the reaction mechanism, the structure and properties of each enzyme. 1) Design and synthesis of transition-state analogue and mechanism-based inhibitors of γ-glutamylcysteine synthetase and γ-glutamyl transpeptidase, the key enzymes in glutathione biosynthesis and its metabolism, respectively. 2) Development of novel asparagine synthetase inhibitors and their application in cancer chemotherapy. 3) Development of intermediate analogue inhibitors of acyl-activating enzyme superfamily that plays pivotal roles in plant hormone homeostasis and secondary metabolite biosynthesis of plants.

Research Activities (Year 2009)

Publications

Ikeuchi H, Meyer ME, Ding Y, Hiratake J, Richards NGJ: A Critical Electrostatic Interaction Mediates Inhibitor Recognition by Human Asparagine Synthetase, *Bioorg. Med. Chem.*, **17**, 6641-6650 (2009).

Ogata M, Hidari KIPJ, Kozaki W, Murata T, Hiratake J, Park EY, Suzuki T, Usui T: Molecular Design of Spacer-*N*-Linked Sialoglycopolypeptide as Polymeric Inhibitors against Influenza Virus Infection, *Biomacromolecules*, **10**, 1894-1903 (2009).

Presentations

Synthesis and Evaluation of Intermediate Analogue Inhibitors of 4-Coumaric Acid: CoA Ligase Involved in Secondary Metabolite Synthesis in Plants, Asai T, Naito Y, Yang Q, Hiratake J, 4th Annual Meeting of Japanese Society for Chemical Biology, Kobe, Japan, 18–19 May 2009.

Crystal Structure of Glutathione Biosynthetic Enzyme from *Streptococcus* sp. Complexed with Sulfoximine-Based Transition-State Analogue Inhibitor, Nakashima Y, Hibi T, Janowiak B, Griffith O, Hiratake J, Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry 2009, Fukuoka, Japan, 27–29 March 2009.

Specific Affinity Chromatography Using Glycone Substrates as Ligand of Cellulases, Kameshima Y, Ogata M, Murata T, Totani K, Hiratake J, Usui T, Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry 2009, Fukuoka, Japan, 27–29 March 2009.

Development of Chemical Tools to Probe the Biosynthesis of Plant-Secondary Metabolites and Auxin Homeostasis -Design and Synthesis of Inhibitors of 4-Coumaroyl CoA Ligase (4CL) and CH3-, Hiratake J, The 2nd Nano-Bio Symposium 2009, Shizuoka, Japan, 6 March 2009 (invited).

Grants

Hiratake J, Development of Chemicals to Control Glutathione Metabolism and Oxidative Stress for Use in Chemical Biology, Grant-in-Aid for Scientific Research (B), 1 April 2007–31 March 2010.

Watanabe B, Development of Novel Chemicals to Regulate Glutathione Biosynthesis, Grant-in-Aid for Young Scientists (Start-up), 1 April 2009–31 March 2011.

Design and Synthesis of γ-Glutamyl Tranpeptidase Inhibitors

Glutathione (γ-Glu-Cys-Gly) plays a central role in detoxification of xenobiotics, and γ-glutamyl tranpeptidase (GGT) is a key enzyme in the metabolism of glutathione. We designed and synthesized transition-state analogue inhibitors highly mimicking glutathione to reveal the substrate-recognition mechanism of GGT. Structure-activity relationships disclosed that human GGT recognizes the stereochemistry of the Cys moiety and the phosphorous atom, and the negative charge at the Gly residue of the inhibitors. On the other hand, *E. coli* GGT showed low specificity particularly with respect to the recognition of the negative charge at the terminal Gly, and the result implied that the primary substrate of *E. coli* GGT is not glutathione. Mass spectrometric analysis showed that the inhibitor (R=Et) binds to the small subunit of GGT covalently in the manner that we anticipated. The crystal structure of a recombinant human GGT revealed that Lys562 strongly interacts with the negative charge at C-terminal Gly of glutathione and the inhibitors.

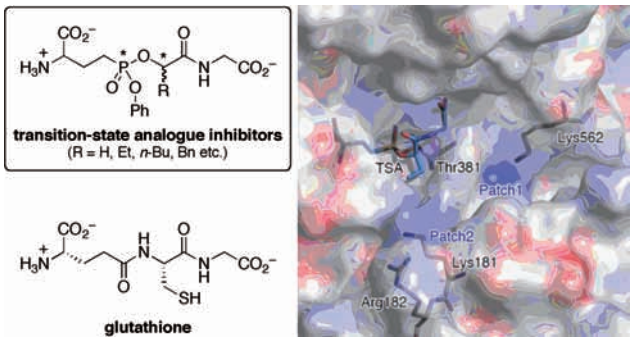

Figure 1. The structure of transition-state analogue inhibitors and substrate binding pocket of human GGT.

Inhibitors Targeting Asparagine Synthetase

Asparagine synthetase (ASNS) catalyzes the synthesis of Asn from Asp in an ATP-dependent manner. The inhibition of ASNS is highly important in enhancing and broadening the efficacy of asparaginase therapy of leukemia and cancer, and we have already developed the first potent *in vitro* ASNS inhibitor (**1**) that suppressed proliferation of asparaginase-resistant cancer cell line at 100-1000 μM. In this study, we aim to increase *in vivo* activity of the original inhibitor by decreasing net negative charge, and synthesized sulfoximino-sulfamide and -sulfamate based inhibitors (**2** and **3**) using rhodium catalyzed coupling of sulfoxide and sulfamide as a key step. Steady-state kinetic characterization of these compounds, however, has revealed the necessity of a localized negative charge on **1** that mimics that of the phosphate group in a key acyl-adenylate reaction intermediate.

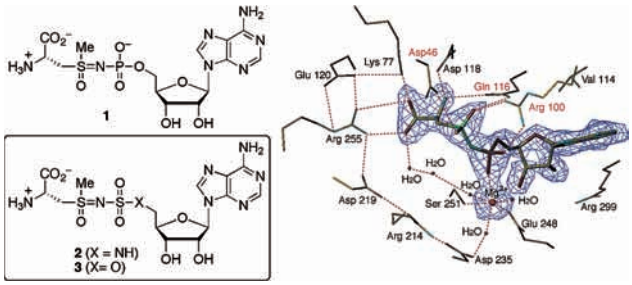

Figure 2. (Left) The structure of the original inhibitor (**1**) and newly synthesized inhibitors (**2** and **3**). (Right) X-Ray crystal structure of *E. coli* ASNS in complex with **1**.

Design of Specific Inhibitors of Acyl-activating Enzymes

Acyl-activating enzymes constitute a large enzyme superfamily that contains a number of such important enzymes as for fatty acid β-oxidation and biosynthesis of plant secondary metabolites. In light of their common mechanistic features involving acyl-adenylate intermediate, we designed and synthesized *N*-acyl adenosyl sulfamide inhibitors to reveal the function of 4-coumaric acid: CoA ligase (4CL), a key enzyme in phenylpropanoid biosynthesis. The synthetic compounds inhibited 4CL *in vitro*, and the substituents on benzene ring significantly affected their potency. Administration of the inhibitors to Arabidopsis caused decrease of the phenylpropanoid contents. This result implied that the inhibitors were uptaken by plant and inhibited 4CL *in vivo*.

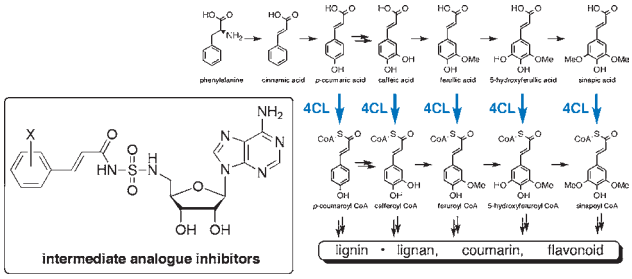

Figure 3. The outline of phenylpropanoid biosynthesis and the structure of intermediate analogue inhibitors.