2.3.12 Laboratory : Chemistry of Molecular Biocatalysts

Member :	Professor	Hiratake, Jun, Dr. Agric. Sci.
	Assistant Professor	Watanabe, Bunta, Dr. Agric. Sci.
	Doctor's program	1
	Master's Program	7
	Researcher	1

A. Research Activities (2009.4-2010.3)

A-1. Main Subjects

a) 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 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.

b) 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 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 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 the lead compound mimicking that of the phosphate group in a key acyl-adenylate reaction intermediate.

c) 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.

A-2.Publications and presentations

a) Publications

Original Papers

- Ikeuchi H., Meyer M.E., Ding Y., Hiratake J. and Richards N.G.J.: A Critical Electrostatic Interaction Mediates Inhibitor Recognition by Human Asparagine Synthetase. Bioorg Med Chem 17; 6641-6650, 2009
- Ogata M., Hidari K.I.P.J., Kozaki W., Murata T., Hiratake J., Park E.Y., Suzuki T. and Usui T.: Molecular Design of Spacer-N-linked Sialoglycopolypeptide as Polymeric Inhibitors against Influenza Virus Infection. Biomacromolecules 10; 1894–1903, 2009

Patents

- Japanese Patent Application No. 2010-032161

b) Conference and seminar papers presented

- 4th Annual Meeting of Japanese Society for Chemical Biology: 1 Presentation
- The 83th Annual Meeting of The Japanese Pharmacological Society: 1 Presentation

A-3.Off-campus activities

Membership in academic societies

- Hiratake, Jun, D.Agric.Sci : Japan Society for Bioscience, Biotechnology, and Agrochemistry Kansai Branch (councillor), Member of "KAGAKU TO SEIBUTSU" editorial board

Research grants

1. Grants-in-aid for Scientific Research(KAKENHI)

- Scientific Research (B) : Hiratake, Jun, D.Agric.Sci : Development chemicals for controlling glutathione metabolism and oxidative stress for use in chemical biology

- Young Scientists (Start-up) : Watanabe, Bunta, D.Agric.Sci : Development of novel chemicals to regulate glutathione biosynthesis

2.Other Research Grants

- Adaptable and seamless technology transfer program through target-driven R&D (JST A-STEP) : Hiratake, Jun, D.Agric.Sci : Application of cell collagen production caused by novel g-glutamyl transpeptidase inhibitors

B.Educational Activities(2009.4-2010.3)

B-1.On-campus teaching

a) Courses given

- Graduate level : Seminar in Molecular Biocatalysts (Hiratake, Watanabe), Laboratory Course in Molecular Biocatalysts (Hiratake, Watanabe), KSI Lecture "Environmental Chemistry and Biochemistry" (Hiratake)

B-2.Off-campus teaching etc.

Part-time lecturer

- Hiratake, J.: Nara Women's University, Faculty of Science (Enzyme Function and Chemistry)