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 superfamly that plays pivotal roles in plant hormone homeostasis and secondary metabolite biosynthesis of plants.

Scope of Research

Research Activities (Year 2009)

Publications


Presentations


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.](image1)

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.](image2)

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 fatty acid β-oxidation and biosynthesis of plant secondary metabolites. In light of their common mechanistic features involving acyl-adenylate intermediates, 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.](image3)