Division of Biochemistry
– Chemistry of Molecular Biocatalysts –

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Scope of Research

Our research focuses on the molecular design and synthesis of specific inhibitors of physiologically important enzymes (biocatalysts). The enzyme inhibitors are used for probing the reaction mechanisms, three-dimensional structures and identifying the physiological roles of the enzymes. The finely designed inhibitors are further elaborated to develop useful bioactive substances that could knockout the specific enzyme *in vivo* and to develop lead compounds for novel pharmaceuticals, agrochemicals and cosmetic ingredients. Our current research includes the design, synthesis and applications of transition-state analog and/or mechanism-based inhibitors of such enzymes as γ-glutamyl transpeptidase, a key enzyme in glutathione metabolism, asparagine synthetase, an important enzyme for cancer chemotherapy, and 4-coumaroyl CoA ligase that plays a pivotal role in the biosynthesis of a vast array of phenylpropanoid in plants. The identification of the genes of hitherto unknown enzymes for biosynthesis of phenylpropanoid volatiles in plants are also pursued to shed light on the detailed reaction mechanisms and the physiological function of the biosynthetic enzymes in plant secondary metabolites.

KEYWORDS

Enzyme Reaction Mechanisms
Transition-State Analogue Inhibitors
Glutathione Homeostasis
Bioactive Substance
Plant Secondary Metabolite Biosynthesis

Selected Publications

Ikeuchi, H.; Ahn, Y. M.; Otokawa, T.; Watanabe, B.; Hegazy, L.; Hiratake, J.; Richards, N. G., A Sulfoximine-based Inhibitor of Human Asparagine Synthetase Kills L-asparaginase-resistant Leukemia Cells, *Bioorg. Med. Chem.*, **20,** 5915-5927 (2012).

Sato, S.; Kawamoto, J.; Sato, S. B.; Watanabe, B.; Hiratake, J.; Esaki, N.; Kurihara, T., Occurrence of a Bacterial Membrane Microdomain at the Cell Division Site Enriched in Phospholipids with Polyunsaturated Hydrocarbon Chains, *J. Biol. Chem.*, **287,** 24113-24121 (2012).

Joyce-Brady, M.; Hiratake, J., Inhibiting Glutathione Metabolism in Lung Lining Fluid as a Strategy to Augment Antioxidant Defense, *Current Enzyme Inhibition*, **7,** 71-77 (2011).

Koeduka, T.; Watanabe, B.; Suzuki, S.; Hiratake, J.; Mano, J.; Yazaki, K., Characterization of Raspberry Ketone/Zingerone Synthase, Catalyzing the Alpha, Beta-Hydrogenation of Phenylbutenones in Raspberry Fruits, *Biochem. Biophys. Res. Commun.*, **412,** 104-108 (2011).

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

Inhibitors Targeting Human Asparagine Synthetase for Cancer Chemotherapy

A standard current clinical protocol for acute lymphoblastic leukemia (ALL) is the use of the enzyme L-asparagine amidohydrolase (ASNase) that catalyzes the hydrolysis of L-Asn to deplete circulating L-Asn in blood. This chemotherapy is unique in that the proliferation of leukemia cells is suppressed by cutting off the supply of nutrient for rapid growth. The efficacy of ASNase chemotherapy, however, is hampered by the emergence of ASNase-resistant leukemic blasts; 10–12% of patients who initially go into remission undergo subsequent relapse. One widely-accepted hypothesis for ASNase resistance is the up-regulation of glutamine- and ATP-dependent asparagine synthetase (ASNS) that enables the leukemia cells to synthesize L-Asn necessary for their growth. Hence the inhibition of human ASNS is highly important in cancer chemotherapy, and we have synthesized a specific inhibitor of human ASNS. According to the proposed reaction mechanism of ASNS in which the β-carboxy group of L-Asp is activated by adenylation followed by nucleophilic substitution by ammonia to yield L-Asn, AMP and PPi, an *N*-adenylated sulfoximine **1** was synthesized as a transition-state analogue inhibitor of ASNS (Figure 1).

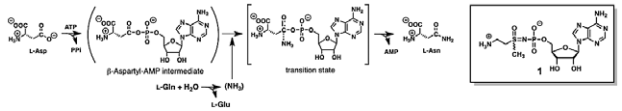

Figure 1. Reaction mechanism of asparagine synthetase and its transition-state analogue 1.

Compound **1** was found to be an extremely potent inhibitor of human ASNS; it inhibited the enzyme in a time-dependent manner with an overall K_i* of 8 nM (Figure 2). Furthermore, the inhibitor **1** suppressed cell proliferation of ASNase-resistant MOLT-4R leukemia cells (Figure 3). The suppression of cell proliferation was dose-dependent with an IC_{50} of ca. 0.1 mM. Surprisingly, compound **1** induced cell death as well as suppression of cell proliferation at higher concentrations. ASNase-sensitive leukemia cells with low ASNS activity were much less sensitive toward compound **1**, suggesting that ASNS in itself was an essential enzyme for leukemia cells and was an important drug target for cancer chemotherapy.

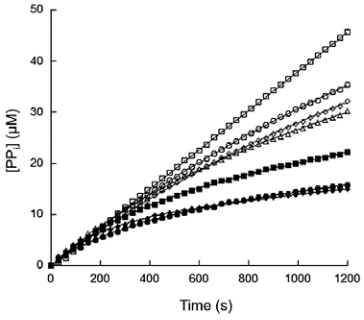

Figure 2. Time-dependent inhibition of human ASNS by compound **1** (0–5 μM).

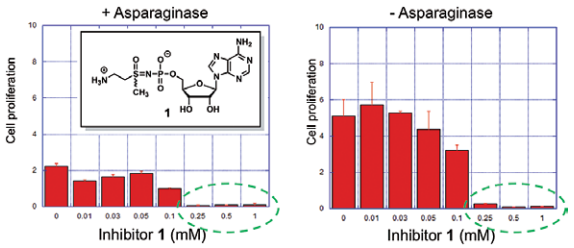

Figure 3. The effect of ASNS inhibitor **1** on the proliferation of ASNase-resistant MOLT-4 leukemia cells in the presence and absence of 1U asparaginase. (Left) MOLT-4 cells with ASNase; (right) MOLT-4R cells without ASNase. Cell proliferation is defined as the number of viable cells after 48 h incubation.

Biosynthetic Pathway of Phenylpropanoid Volatiles in Plants

Phenylpropanoid volatiles including raspberry ketone and zingerone, are produced in plants, and are characteristic constituents in many fruits and spices, and floral bouquet. Double bond reductases, which recognize secondary metabolites such as (2*E*)-nonenal, (+)-pulegone, and furaneol derivatives, have been identified, but little is known about the presumably similar enzymes responsible for the biosynthesis of phenylpropanoid volatiles. By using a homology-based PCR, we isolated a cDNA encoding raspberry ketone/zingerone synthase (RZS) from raspberry fruits. The protein encoded by this cDNA can catalyze the NADPH-dependent reduction of 4-hydroxybenzalacetone and 3-methoxy-4-hydroxybenzalacetone to raspberry ketone and zingerone, respectively. The substrate specificity experiments suggested that RZS strictly discriminates the methyl group in the side chain of substrates. We are currently investigating other double-bond reductases that may be responsible for the branching of the biosynthetic pathway of phenylpropanoid volatiles in petunia flowers.

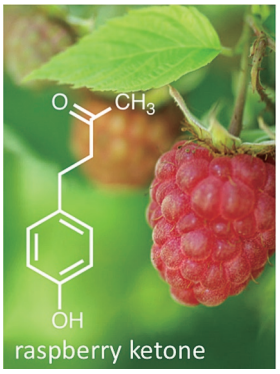

Figure 4. Characteristic aroma of raspberry fruits.