Division of Biochemistry
- Chemistry of Molecular Biocatalysts -

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Scope of Research

Our research covers the comprehensive understanding of the physiological roles of biocatalysts (enzymes) as well as the reaction mechanism and specificity of each enzyme. 1) Studies on diglycosidases hydrolyzing the β-glycosidic bond between disaccharides and aglycons. 2) Molecular basis of the floral aroma formation in oolong tea. 3) Design and synthesis of transition-state analogue and mechanism-based inhibitors of γ-glutamyltranspeptidase. 4) Directed evolutional studies of *Pseudomonas* lipase. 5) Studies on the activation/inactivation process of plant hormones. 6) Molecular mechanism of regulation of phenylpropanoid pathway in plants.

Research Activities (Year 2006)

Presentations

Inhibitors of IAA-Amino Acid Conjugate Synthetases and Hydrolases as Chemical Probes to Study IAA Homeostasis, Tai LH, Hiratake J, Shimizu B, Mizutani M, Sakata K, 11th IUPAC International Congress of Pesticide Chemistry, Kobe, 8 August.

X-ray Crystallography of a Diglycosidase from Plants, Saino H, Mizutani M, Hiratake J, Shimizu T, Kato H, Sakata K, 2006 Annual Meeting, Jpn. Soc. Biosci. Biotech. Agrochem., 27 March.

Glucosylation of Scopoletin in *Arabidopsis* Roots, Kai K, Shimizu B, Yamaguchi H, Mizutani M, Sakata K, 2006 Annual Meeting, Jpn. Soc. Plant Physiol., 20 March.

Grants

Sakata K, Studies on Catalytic Mechanism of Disaccharide-Specific Glycosidases and Evolution of Plant β-Glucosidases, Grant-in-Aid for Scientific Research (B) (2), 1 April 2004–31 March 2007.

Hiratake J, Bio- and Organic Chemical Studies on Plant Glycosidases by Using β-Glycosylamidine Derivatives as Tools, Grant-in-Aid for Scientific Research (B) (2), 1 April 2004–31 March 2007.

Hiratake J, Chemical Tools for Probing into IAA Homeostasis – Design and Synthesis of Inhibitors of IAA-Amino Acid Conjugate Hydrolases and Synthathases, Grant-in-Aid for Exploratory Research, 1 April 2005–31 March 2006.

Design, Synthesis and Evaluation of γ-Glutamyl Transpeptidase Inhibitors

γ-Glutamyl transpeptidase (GGT) catalyzes the hydrolysis of glutathione and its *S*-conjugates and plays a pivotal role in glutathione metabolism. GGT is involved in important biological events such as drug resistance and metastasis of cancer cells by detoxification of xenobiotics and reactive oxygen species, and is also implicated in physiological disorders such as Parkinson's disease, cardiovascular diseases and asthma through glutathione metabolism and leukotriene biosynthesis. We designed and synthesized a series of γ-phosphono diester glutamate analogues as mechanism-based inhibitors of GGT. The phosphonates reacted with the N-terminal catalytic Thr residue of GGT to cause facile enzyme inactivation. A series of the phosphonate inhibitors were synthesized to probe successfully the active-site geometry of human GGT, where a specific residue in the Cys-Gly binding site played a critical role in recognizing the C-terminal carboxy group of glutathione and its conjugates. The phosphonate diesters were highly selective towards GGT and did not inhibit glutamine amidotransferases, the important enzymes for purine and pyrimidine biosynthesis. The phosphonate diester-based GGT inhibitors serve as drug leads and biological probes that gain insight into the hitherto undefined physiological roles of GGT and the relationships between GGT and a variety of diseases.

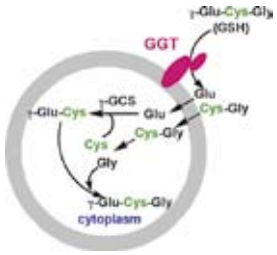

Figure 1. Physiological role of GGT.

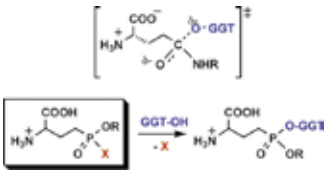

Figure 2. Proposed transition state of the reaction catalyzed by GGT and γ-phosphono diester glutamate analogues as mechanism-based inhibitors of GGT.

Chemical Inhibitors for Abscisic Acid Catabolism

A plant hormone, abscisic acid (ABA), regulates many important physiological processes including adaptive responses to abiotic stresses. The main catabolic pathways involve hydroxylation of the C-8' position of ABA by cytochrome P450 monooxygenases (P450), and we recently identified CYP707As as ABA 8'-hydroxylase. Plant growth retardants (PGRs) are known to reduce the shoot growth of plants by inhibiting P450s in gibberellin biosynthesis. We performed detailed analyses of the inhibitory effects of PGRs on *Arabidopsis* ABA 8'-hydroxylase. Uniconazole-P was found to be a strong competitive inhibitor (K_i = 8.0 nM) of ABA 8'-hydroxylase. Uniconazole-P-treated *Arabidopsis* plants showed enhanced drought tolerance (Figure 3). In uniconazole-P-treated plants, endogenous ABA levels increased 2-fold as compared with the control. Thus, specific inhibitors of ABA catabolism can manipulate ABA homeostasis in plants and are potentially very useful tools for cellular and molecular investigations in the field of plant physiology as well as for potential agricultural chemicals.

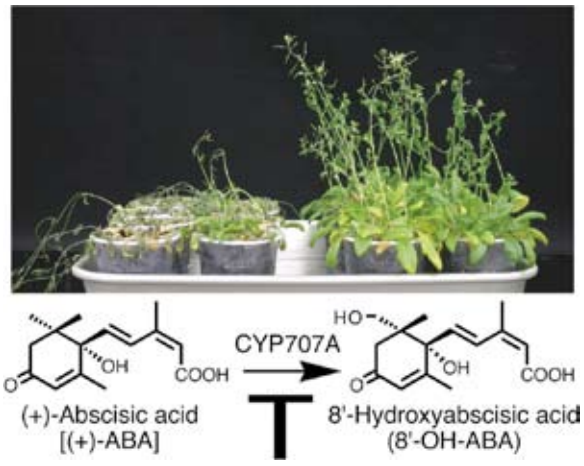

Figure 3. Inhibitors for ABA catabolism can manipulate ABA homeostasis and enhance plant drought tolerance.

Mizutani M, Construction of Plant Oxygenase Library and Its Functional Characterization, Grant-in-Aid for Scientific Research (C) (2), 1 April 2006–31 March 2008.

Awards

Mizutani M, The JSCRP Award for the Encouragement of Young Scientists, "Biochemical Studies on Cytochrome P450 Monooxygenases in Abscisic Acid and Plant Steroid Metabolism", The Japanese Society for Chemical Regulation of Plants, 30 October 2006.

Ohnishi T, Poster Award, "Functional Characterization of *Arabidopsis* CYP90C1 and CYP90D1 Encoding Brassinosteroids C-23 Hydroxylases". 8th International Symposium on Cytochrome P450 Biodiversity and Biotechnology, Swansea, UK, 27 July 2006.

Ohnishi T, Poster Award, "Biochemical Characterization of Brassinosteroid C-3 Oxidase", The 41th Annual Meeting of the Japanese Society for Chemical Regulation of Plants, Osaka, Japan, 30 October 2006.