Division of Biochemistry
- Chemistry of Molecular Biocatalysts -

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Assoc Prof
HIRATAKE, Jun
(D Agr)

Assist Prof
MIZUTANI, Masaharu
(D Agr)

Assist Prof
SHIMIZU, Bun-ichi
(D Agr)

PD
CHO, Jeong-Yong
(D Agr)

PD
OHNISHI, Toshiyuki
(D Agr)

PD
HAN, Li-You
(D Agr)

Students

NAKAGAWA, Yuichi (D3)
SAINO, Hiromichi (D3)
FUKATANI, Yoshimi (M2)
HORITA, Junko (M2)
KAWAMURA, Naohiro (M2)
TAKEUCHI, Yoshinori (M2)
YAMAMOTO, Ryotaro (M2)
IKEUCHI, Hideyuki (M1)
KAWABE, Ayami (M1)
NAITO, Yoshiyuki (M1)
NAKAJIMA, Mado (M1)
MATSUMOTO, Seitaro (M1)

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) Development of intermediate analogue inhibitors of acyl-activating enzyme superfamily that plays pivotal roles in plant hormone homeostasis. 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 coumarin biosynthesis in plants.

Research Activities (Year 2007)

Publications

Han L, Hiratake J, Kamiyama A, Sakata K: Design, Synthesis and Evaluation of γ-Phosphono Diester Analogues of Glutamate as Highly Potent Inhibitors and Active Site Probes of γ-Glutamyl Transpeptidase, *Biochemistry*, **46**, 1432-1447 (2007).

Ahn Y O, Saino H, Mizutani M, Shimizu B, Sakata K: Vicianin Hydrolase is a Novel Cyanogenic β-Glycosidase Specific to β-Vicianoside (6-*O*-α-L-Arabinopyranosyl-β-D-Glucopyranoside) in Seeds of *Vicia angustifolia*, *Plant Cell Physiol*, **48**, 938-947 (2007).

Nakagawa Y, Hasegawa A, Hiratake J, Sakata K: Engineering of *Pseudomonas aeruginosa* Lipase by Directed Evolution for Enhanced Amidase Activity: Mechanistic Implication for Amide Hydrolysis by Serine Hydrolases, *Protein Eng Des Sel*, **20**, 339-346 (2007).

Presentation

The Campestanol-Independent Pathway of Brassinosteroid Biosynthesis, Mizutani M, 19th International Conference on Plant Growth Substances, Mexico, 22 July 2007.

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, Development of Chemicals to Control Glutathione Metabolism and Oxidative Stress for Use in Chemical Biology, Grant-in-Aid for Scientific Research (B) (2), 1 April 2007–31 March 2010.

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.

Mechanism of Disaccharide-Glycone Specificity in β-Primeverosidase Is Revealed by the Crystal Structure in Complex with β-Primeverosylamidine

β-Primeverosidase (PD) is a family 1 glycosidase catalyzing the hydrolysis of β-primeverosides (6-*O*-β-D-xylopyranosyl-β-D-glucopyranosides) to release a disaccharide primeverose (Figure 1a). To investigate how PD recognizes the disaccharide moiety of β-primeverosides, we determined the crystal structure of PD in complex with β-primeverosylamidine (Figure 1b) as a chemical probe at a resolution of 1.8 Å (Figure 1c). The shape of the substrate-binding pocket consisting of subsites –2, –1, and +1 is like a funnel approximately 18 Å deep, and its entrance is 14 Å long and 10 Å wide. In this pocket, the glycosidic nitrogen of β-primeverosylamidine interacts with the oxygen atom of Glu203 (acid/base) at a distance of 2.7 Å, and the oxygen atom of Glu416 (nucleophile) interacts with C1 of glucose at a distance of 3.2 Å. All the hydroxy groups on the primeverosyl moiety make hydrogen bonds with surrounding residues in subsites –2 and –1. The interaction with the glucose moiety in subsite –1 is almost identical to that in β-glucosidases. The subsite –2 is constituted of six amino acids, Val386, Phe389, Glu470, Ser473, Gln477, and Phe479, and these residues are crucial and responsible for the strict specificity of PD toward the xylosyl moiety of β-primeverosides.

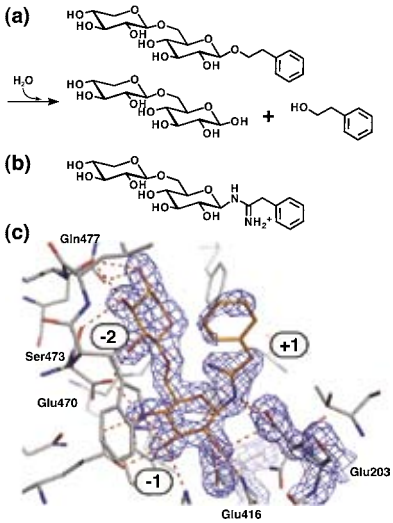

Figure 1. Crystal structure of PD in complex with β-primeverosylamidine. (a) The reaction scheme of PD. (b) β-primeverosylamidine. (c) Conformation of β-primeverosylamidine in the substrate-binding pocket of PD.

Biosynthetic Pathway of Coumarins in Plants

Coumarins are often found in the plant kingdom. They are involved in the plant defense due to their antimicrobial and antioxidative activities. They are originated from the phenylpropanoid pathway *via ortho*-hydroxylation of cinnamates, *cis-trans* isomerization of the side chain, and lactonization. The *ortho*-hydroxylation step is a key step, because it is the branching point from the general phenylpropanoid pathway. We explored coumarin biosynthesis using *Arabidopsis thaliana*, which accumulates scopolin, a glucoside of scopoletin (7-hydroxy-6-methoxycoumarin) in their roots. *Ortho*-hydroxylase of cinnamates was examined in the oxygenase families in Arabidopsis, and one of the candidate genes in the 2-oxoglutarate dependent dioxygenase family was designated as *F6'H1*. The T-DNA insertion mutants of F6'H1 exhibited severe reduction in the scopoletin/scopolin level in the roots. The recombinant F6'H1 protein exhibited *ortho*-hydroxylase activity for feruloyl-CoA to form 6'-hydroxyferuloyl-CoA. These results indicate that the 2-oxoglutarate dependent dioxygenase is the pivotal enzyme in *ortho*-hydroxylation of feruloyl-CoA in scopoletin biosynthesis. Adding to *F6'H1*, we also identified several genes involved in scopolin biosynthesis in Arabidopsis.

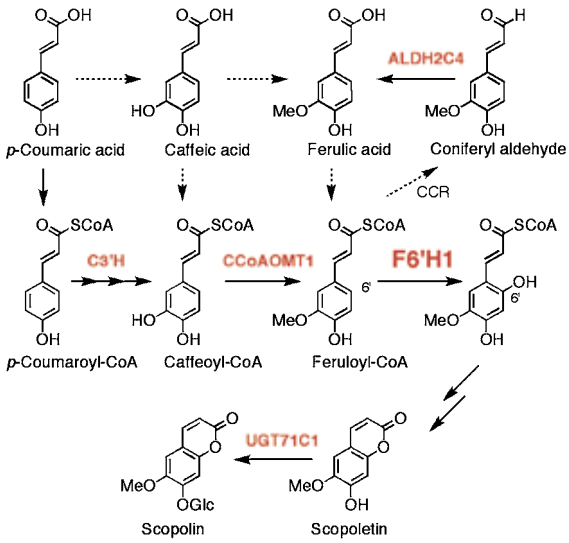

Figure 2. Biosynthetic pathway of scopoletin in *Arabidopsis thaliana.*

Awards

Ohnishi T, Watanabe B, Sakata K, Mizutani M, Paper Award, Award for Excellence to Authors Publishing in Bioscience, Biotechnology, and Biochemistry in 2006, "CYP724B2 and CYP90B3 Function in the Early C-22 Hydroxylation Steps of Brassinosteroid Biosynthetic Pathway in Tomato", Japan Society for Bioscience, Biotechnology, and Agrochemistry (NIPPON NOGEI-KAGAKU KAI), 24 March 2007.

Takeuchi Y, Poster Award, "Chemical Tools to Control IAA Homeostasis – IAA-Amino Acid Synthathase (GH3) Inhibitors and their *in vivo* Activiies –", The 42nd Annual Meeting of The Japanese Society for Chemical Regulation of Plants, Shizuoka, Japan, 30 October 2007.