

ELUCIDATION, GENERATION, AND APPLICATION OF ENZYME FUNCTIONS

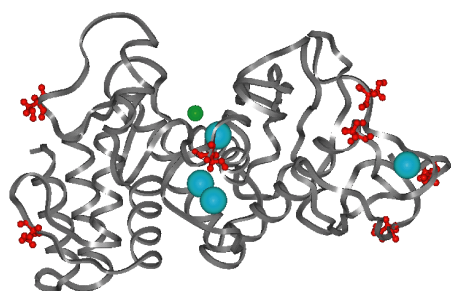
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Enzymes are proteins having catalytic activity. Biological reactions are accelerated and accurately controlled by enzymes in organisms. On the other hand, enzymes are widely used in industry. We are aiming at elucidating the relationship between structure and function of enzymes at a molecular level and developing new methods for application of enzymes in food and medical technologies by generating new enzyme functions and controlling enzyme reactions. We are focusing our activities mainly on the following subjects.

Tailoring Functions of an Enzyme Used for Producing Food Materials

Thermolysin (TLN) is a metalloproteinase used for synthesis of an artificial sweetener, aspartame. We have reported marked activation of TLN by high concentrations of salts. Extensive studies are underway to generate TLN with higher activity by a genetically engineering technique.

Thermolysin

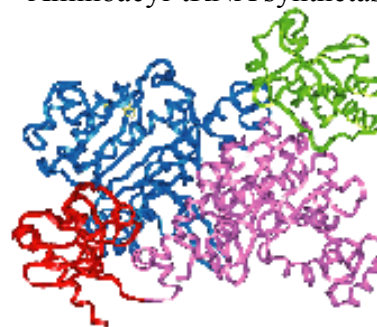


Green, zinc;
blue, calcium;
red, mutated sites.

Basic Research Aimed at Designing Useful Enzymes

Aminoacyl-tRNA synthetase (aaRS) guarantees the fidelity of translation of genetic information into protein. It has acquired the highest degree of substrate recognition during evolution of life. The elucidation of this recognition mechanism might provide information required for designing enzymes.

Aminoacyl-tRNA synthetase



Blue and purple, catalytic domain;
red and green, tRNA anticodon-binding domain.

Reaction Mechanism of an Enzyme Involved in Tumor Metastasis

Matrix metalloproteinase (MMP) is a metalloproteinase that degrades the extracellular matrix and takes part in tumor metastasis and invasion. We have analyzed the reaction mechanism of MMP-7 and the inhibitory effects of green tea catechins and lignans on MMP-7 activity. We are exploring new inhibitors based on the reaction mechanism.

Tailoring Functions of an Enzyme Used for Clinical Diagnosis

Reverse transcriptase (RT) is an enzyme which is indispensable as a tool for research in molecular biology and diagnosis of RNA virus. RTs from avian myeloblastosis virus (AMV) and Moloney murine leukaemia virus (MMLV) have been the most extensively used. We are in process to improve their activities and stabilities by site-directed mutagenesis.

Key Words

Enzyme, enzyme chemistry, reaction mechanism, spectroscopy, food industry, amylase, protease

Recent Publications

Effects of site-directed mutagenesis of Asn116 in the β -hairpin of the N-terminal domain of thermolysin on its activity and stability

Menach E, Yasukawa K, Inouye K (2012) J Biochem in press

Improving the thermal stability of avian myeloblastosis virus reverse transcriptase α subunit by site-directed mutagenesis

Konishi A, Yasukawa K, Inouye K (2012) Biotechnol Lett in press

A possibility of a water molecule bound to the main chain of human matrix metalloproteinase 7 (MMP-7) as the ionizable group responsible for pK_c at the alkaline side in MMP-7 activity

Morishima A, Yasukawa K, Inouye K (2012) J Biochem 151: 501-509

Interaction of 8-anilino-naphthalene 1-sulfonate (ANS) and human matrix metalloproteinase 7 (MMP-7) as examined by MMP-7 activity and ANS fluorescence

Samukange V, Yasukawa K, Inouye K (2012) J Biochem 151: 533-540

Stabilization of bovine intestine alkaline phosphatase by sugars

Sekiguchi S, Hashida Y, Yasukawa K, Inouye K (2012) Biosci Biotechnol Biochem 76: 95-100

Effects of polyethylene glycol on bovine intestine alkaline phosphatase activity

Sekiguchi S, Yasukawa K, Inouye K (2011) Biosci Biotechnol Biochem 75: 2132-2137

Thermodynamic analysis of ionizing groups involved in the catalytic mechanism of human matrix metalloproteinase 7 (MMP-7)

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Comparison of the thermal stabilities of the $\alpha\beta$ heterodimer and the α subunit of avian myeloblastosis virus reverse transcriptase

Konishi A, Nemoto D, Yasukawa K, Inouye K (2011) *Biosci Biotechnol Biochem* 75:1618-1620

Analysis of the mechanism of inhibition of human matrix metalloproteinase 7 (MMP-7) activity by green tea catechins

Miyake T, Yasukawa K, Inouye K (2011) *Biosci Biotechnol Biochem* 75: 1564-1569

Tyr219 of human matrix metalloproteinase 7 (MMP-7) is not critical for catalytic activity, but is involved in the broad pH-dependence of the activity

Muta Y, Inouye K (2011) *J Biochem* 150:183-188

Activation of matriptase zymogen

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Narita Y, Inouye K (2011) *Food Chem* 127:1532-1539

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Menach E, Yasukawa K, Inouye K (2010) *Biosci Biotechnol Biochem* 74: 2457-2462

Expression in *Escherichia coli*, refolding, and purification of the recombinant mature form of human matrix metalloproteinase 7 (MMP-7)

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The structural requirements of matriptase in its ectodomain release in polarized epithelial cells

Tsuzuki S, Murai N, Miyake Y, Inouye K, Fushiki T (2010) *Biosci Biotechnol Biochem* 74: 1295-1297

Identification of the matriptase second CUB domain as the secondary site for interaction with hepatocyte growth factor activator inhibitor type-1

Inouye K, Tsuzuki S, Yasumoto M, Kojima K, Mochida S, Fushiki T (2010) *J Biol Chem* 285: 33394-33403

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Matriptase does not require hepatocyte growth factor activator inhibitor type-1 for activation in an epithelial cell expression model.

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Kusano M, Yasukawa K, Inouye K (2010) J Biotechnol 147: 7-16

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Effects of thermal treatment on the coagulation of soy proteins induced by *subtilisin Carlsberg*

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