

## YAMAMOTO LABORATORY (July 1960~)

Head: Dr. Tatsuo Yamamoto

Since the establishment of this laboratory in 1960, biochemical aspects of amylolytic enzymes, cell membrane transport and amino acids metabolism in micro-organisms have been investigated here as follows.

### I. Studies on amylolytic enzymes

(1) The effect of lipids on amylolytic enzyme production<sup>1)</sup>

It was found that amylolytic enzymes of *Endomyces* sp. was produced stimulative by the addition of rice-bran to the medium, and that lipids in rice-bran were effective. Fatty acids derived from the lipids showed the same stimulative effect. The mechanism of the stimulation was discussed.

(2) Amylolytic enzymes in wheat seed<sup>2)</sup>

Changes of amylolytic enzyme activities during storage and other industrial processes of wheat seeds were investigated and it was demonstrated that the embryo releases a hormone which triggers the release of amylolytic, proteolytic and cytolytic enzymes from the aleurone cells. The similar function was caused also by the addition of gibberellic acid. The relationship between gibberellic acid and the development of enzymes during wheat malting was elucidated.

(3) New characteristic alpha-amylase in sweet potato juice<sup>3,11)</sup>

A new amylolytic enzyme which the dextrinizing activity places with the  $\alpha$ -amylase, was demonstrated in raw sweet potato juice. Among its unusual characteristics were high optimum activity temperature (70°-75°), heat stability and low activity at ordinary temperature.

Freshly harvested sweet potatoes contain relatively small amounts of this enzyme, which increases about sixfold after nine months' storage.

### II. Studies on cell membrane transport in yeast<sup>4,5)</sup>

The function of the surface structure of yeast cells was investigated from the viewpoint of sugar transport. Glucose and sugar phosphates could not penetrate into the cell without the action of hexokinase and phosphatase, which located on the surface structure of the cell. Glucose incorporation was performed under the active state of hexokinase, and sugar phosphates were incorporated after the substrates had been decomposed to sugar and inorganic phosphate by phosphatase reaction. Sugar moiety was transported through the membrane by the aid of hexokinase, and inorganic phosphate remained outside of the cell.

### III. Studies on metabolism of amino acids

(1) Metabolism of theanine and the related compounds<sup>6-9)</sup>

Some strains of *Pseudomonas* were found capable of utilizing L-theanine and D-theanine as a sole nitrogen and carbon sources. L-Theanine and D-theanine were hydrolyzed by the enzyme from *Pseudomonas aeruginosa* to yield stoichiometrically L-glutamate and D-glutamate, respectively, and ethylamine, which were isolated from the reaction mixture, and identified.

Theanine hydrolase was purified approximately 200-fold. It was shown that the activities of L-theanine hydrolase, D-theanine hydrolase and the heat-stable L-glutamine hydrolase and D-glutamine hydrolase are ascribed to a single enzyme, which may be regarded as a  $\gamma$ -glutamyltransferase from the point of view of the substrate specificity and the other properties. This theanine hydrolase catalyzed the transfer of  $\gamma$ -glutamyl moiety from the substrates and glutathione to hydroxylamine.

L-Glutamine and D-glutamine were hydrolyzed by theanine hydrolase and also the heat-labile enzyme whose properties resemble the common glutaminase.

(2) L-Lysine- $\alpha$ -ketoglutaric acid transaminase<sup>10)</sup>

Transaminase activity between L-lysine and  $\alpha$ -ketoglutaric acid was confirmed to be present in the cell-free extracts of *Flavobacterium fuscum*, *Fl. flavescens* and *Achromobacter liquidum*. The product from L-lysine was isolated from the reaction mixture by ion exchanger column chromatography, and identified as  $\Delta^1$ -piperidine-6-carboxylic acid, which is formed from  $\alpha$ -aminoadipic- $\delta$ -semialdehyde by an intramolecular dehydration. The latter compound is derived direct from L-lysine by the transaminase reaction. Some properties of the enzyme were investigated.

### Publications

1. T. Yamamoto, F. Hattori and M. Takatsu: The effect of lipids on amylolytic enzyme production by *Endomyces* sp. *Bull. Inst. Chem. Res., Kyoto Univ.*, **42**, 252 (1964).
2. J. A. Johnson, J. R. Fleming and M. Ikemiya: Enzymes systems important in wheat utilization. *Proc. Second National Conference on Wheat Utilization Research*, 229 (1963).
3. M. Ikemiya and H. H. Deobald: New characteristic alpha-amylase in sweet potatoes. *J. Agr. Food Chem.*, **14**, 237 (1966).
4. Y. Kariya, T. Yamamoto and S. Nagasaki: Some observations on cell membrane transport in yeasts. *Physiology and Ecology*, **11**, 183 (1963).
5. Y. Kariya, S. Nagasaki and T. Yamamoto: Some observations on cell membrane transport in yeasts. *Bull. Inst. Chem. Res., Kyoto Univ.*, **43**, 221 (1965).
6. K. Soda, K. Uchiyama and K. Ogata: Metabolism of L-theanine, D-theanine and the related compounds in bacteria. Part I. Bacterial and enzymatic hydrolysis of L- and D-isomers of theanine and identification of products. *Agr. Biol. Chem.*, **30**, 541 (1966).
7. K. Soda, K. Uchiyama and K. Ogata: Metabolism of L-theanine, D-theanine and the related compounds in bacteria. Part II. Purification and properties of the enzyme hydrolyzing L-theanine, D-theanine and the related compounds. *Agr. Biol. Chem.*, **30**, 547 (1966).
8. K. Soda, K. Uchiyama and K. Ogata: Enzymatic hydrolysis of theanine and the related compounds. *Bull. Inst. Chem. Res., Kyoto Univ.*, **43**, 480 (1966).
9. K. Soda, K. Uchiyama and K. Ogata: Studies on enzymatic hydrolysis of theanine and the related compounds. *Amino Acids and Nucleic Acids*, **14**, 49 (1966).
10. K. Soda, H. Misono and T. Yamamoto: L-Lysine- $\alpha$ -ketoglutaric acid transaminase reaction: Identification of the product from L-lysine. *Agr. Biol. Chem.*, **30**, 944 (1966).

### Review

- T. Yamamoto and T. Osumi: Petroleum microbiology. *J. Japan Petroleum Inst.*, **9**, 40 (1966).