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LABORATORY OF MICROBIAL BIOCHEMISTRY

Head: Dr. Tatsuo Yamamoto

This laboratory was established by Prof. H. Katagiri in 1942 to study the chemical aspects of general and applied microbiology, and was succeeded by Prof. T. Yamamoto in 1960. Dr. K. Soda, Dr. Y. Kariya, Dr. T. Osumi, and Dr. H. Misono have worked as an associate professor and a research associate, respectively. Microbial metabolism of amino acids and nitro compounds, structure, function, and regulation of pyridoxal phosphate enzymes, antitumore enzymes, and utilization of fermentation energy of yeast for biosynthetic processes have been investigated here during the last decade.

I. Studies on Amino Acid Racemases[5,54]

1) Amino Acid Racemase with Low Substrate Specificity[1,14,15,18,26]

A new amino acid racemase catalyzing racemization of either D- or L-isomer of α-aminobutyrate and leucine was found in a cell-free extract of *Pseudomonas striata*, which was isolated from soil and identified. This racemase was purified to homogeneity and crystallized to elucidate physicochemical and enzymological properties. The enzyme catalyzes the racemization of various amino acids including lysine, ornithine and ethionine, and contains 2 moles of pyridoxal phosphate.

2) Arginine Racemase[3,4,16,25,31,38]

Arginine racemase was discovered in the extract of *Pseudomonas graveolens*, purified to homogeneity, and crystallized. The enzyme contains 4 moles of pyridoxal phosphate per mole of enzyme (Mw=167,000) and catalyzes not only the racemization of a variety of amino acids, *e.g.*, lysine, arginine, ornithine and citrulline, but also the transamination of ornithine with pyruvate. The regulation of arginine racemase by transamination between the enzyme-bound pyridoxal phosphate (or pyridoxamine phosphate) and an amino acid such as ornithine (or an α-keto acid, *e.g.*, pyruvate) was demonstrated.

3) Alanine Racemase[70]

Alanine racemase of *Bacillus subtilis var. aterrimus* was purified approximately 1,000-fold. The enzyme is exclusively specific for alanine. It was shown that the enzyme requires pyridoxal phosphate as a cofactor and flavin compound have no effect on the enzyme activity.

II. Studies on Metabolism of Lysine and Its Analogues[45]

1) L-Lysine-α-ketoglutarate ε-Aminotransferase[7–10,17,27,36]

L-Lysine-α-ketoglutarate ε-aminotransferase was purified to homogeneity and

crystallized from *Achromobacter liquidum*. The enzyme catalyzes transfer of the terminal amino groups of L-lysine, L-ornithine and *S*-(β-aminoethyl)-L-cysteine to α-ketoglutarate to yield Δ^1-piperideine-6-carboxylate, Δ^1-pyrroline-5-carboxylate, and 4-thia-Δ^1-piperideine-6-carboxylate, respectively. The enzyme contains two moles of pyridoxal phosphate per mole of enzyme (Mw=116,000). One of them with λmax at 420 nm plays an essential role in the catalytic reaction. The other pyridoxal phosphate (λmax: 340 nm) is bound in a catalytically inactive form.

2) **L-Lysine Decarboxylase**[11,28,53]

Crystalline L-lysine decarboxylase (Mw=1,000,000) was obtained from *Bacterium cadaveris* grown in the media containing L-lysine as an inducer. δ-Hydroxylysine and *S*-(β-aminoethyl)-L-cysteine are decarboxylated by the enzyme.

3) **Bacterial Metabolism of Lysine Analogues**[13,20,22,62,65,68,73,78,84]

Aerobacter aerogenes was found capable of growing well in the medium containing *S*-(β-aminoethyl)-L-cysteine, a metabolic antagonist of L-lysine, as a nitrogen source. The main metabolic products from *S*-(β-aminoethyl)-L-cysteine was isolated and identified as *S*-(β-*N*-acetylaminoethyl)-L-cysteine and *S*-(β-*N*-acetylamino)-α-keto-mercaptopropionate. Terminal amino group of *S*-(β-aminoethyl)-L-cysteine is acetylated by *N*-acetyltransferase to yield *S*-(β-*N*-acetylaminoethyl)-L-cysteine, and then the acetylated compound is readily deaminated by L-amino acid oxidase to form *S*-(β-*N*-acetylaminoethyl)-α-keto-mercaptopropionate. The enzyme catalyzing the first step, *S*-(β-aminoethyl)-L-cysteine ω-*N*-acetyltransferase was purified to homogeneity from *A. aerogenes*. In addition to *S*-(β-aminoethyl)-L-cysteine, its D-enantiomer, sulfoxide, sulfone and a higher homologue, an oxygen analogue of lysine, and L- or D-lysine also accept an acetyl group. Acetyl-Co A is the exclusive acyl donor.

III. Studies on Pyridoxal Phosphate Enzymes[12]

1) **Kynureninase**[29,33,35,49,50,58,59]

Inducible kynureninase was purified to homogeneity and crystallized from *Pseudomonas marginalis*. One mole of pyridoxal phosphate is bound per mole of enzyme (Mw=100,000). The enzyme can act also as an α-aminotransferase of high substrate specificity to regulate the enzyme activity by interconversion of the coenzyme moiety.

2) **Methioninase**[89]

L-Methioninase, which catalyzes the conversion of L-methionine to α-ketobutyrate, methanthiol and ammonia, was purified to homogeneity from *Pseudomonas ovalis*. The enzyme (Mw=180,000) catalyzes both α,γ- and α,β-elimination reactions of several derivatives of L-methinine and L-cysteine.

3) **D-Amino Acid Aminotransferase**[61,69,72,76]

D-Amino acid aminotransferase, purified to homogeneity and crystallized from *Bacillus sphaericus*, has a molecular weight of about 60,000 and consists of two identical subunits. The enzyme contains 2 moles of pyridoxal phosphate per mole of enzyme and catalyzes transamination of various D-amino acids with α-keto acids. One mole of

pyridoxal phosphate is bound to the inactive form of enzyme, semiapoenzyme.

4) **Taurine-α-ketoglutarate Aminotransferase**[34,38,39,42,43,60]

Taurine-α-ketoglutarate aminotransferase was discovered in the cell-free extracts of *Achromobacter superficialis* and *A. polymorph.* The crystalline enzyme (Mw=156,000) was obtained from *A. superficialis.* Four moles of pyridoxal phosphate are bound per mole of enzyme. The enzyme inactivated by treatment with ammonium sulfate is activated by incubation with pyridoxal phosphate at 45–60°.

5) **Taurine-Pyruvate Aminotransferase**[51,52]

A new aminotransferase which catalyzes transamination between taurine and pyruvate was found in the cell-free extract of *Pseudomonas* sp. isolated from soil.

6) **Aspartate Aminotransferase**[77,83]

Aspartate aminotransferase (Mw=80,000) was purified to homogeneity and crystallized from *Pseudomonas striata.* Its enzymological properties are compared to those of mammalian enzymes.

7) **Kynurenine Aminotransferase**[74]

Kynurenine aminotransferase occurs in several strains of yeasts. The enzyme was partially purified from *Hansenula schneggii* and some of its properties were investigated.

IV. Studies on Biostereochemistry of Histidine Metabolism[57]

The stereochemistry of deamination of histidine which is catalyzed by histidase of *Pseudomonas striata* was assigned to be a stereospecific *trans*-elimination including pro-R proton liberation from the C-3 position of L-histidine.

V. Studies on Antineoplastic Enzymes[23,30,37,40,41,44,47,48,87,92]

L-Leucine dehydrogenase, which was purified to homogeneity and crystallized from *Bacillus sphaericus,* was shown to be highly inhibitory to Ehrlich ascites carcinoma *in vivo.* Tumor-bearing mice treated with leucine dehydrogenase showed a progressive increase in life span with increasing dose of the enzyme. An isozyme of glutaminase from *Pseudomonas aeruginosa,* glutaminase A, also resulted in a significant prolongation of survival times, while glutaminase B did not significantly change life span.

VI. Studies on Biochemistry of Alkyl Nitro Compounds[80,82,86]

Various strains of bacteria, yeast and fungi were capable of growing in a medium containing nitroethane as a nitrogene source. Nitroalkanes are oxidatively denitrified to form nitrite by cells and extracts of organisms grown in the medium. A nitroalkane-oxidizing enzyme was purified to homogeneity from *Hansenula mrakii* which was most active in assimilating nitroethane. The enzyme is a non-heme iron flavoprotein which catalyzes the reaction of molecular oxygen and 2-nitropropane yielding

nitrite and acetone, into which molecular oxygen was incorporated. In addition to 2-nitropropane, nitroethane, 3-nitro-2-pentanol, and 1-nitropropane are oxidatively denitrified by the enzyme, 2-nitropropane dioxygenase.

VII. Studies on Utilization of Fermentation Energy of Yeast for Biosynthetic Processes[55,56,63,67,68,71,79,81,88,90,91,93]

This study is concerned with the utilization of fermentation energy of yeast for the phosphorylation of biologically important organic compounds. The principle of energy generation is based on the Harden-Young effect of fermentation of glucose which is preceded by the addition of high concentration of inorganic phosphate ions to the reaction mixture. Air-dried cells of baker's yeast and other yeasts were employed as energy generating system, which was coupled with the energy-requiring system of the yeast or with enzymatic phosphorylating systems of the other organisms.

Various nucleoside triphosphates and glucosamine 6-phosphate were formed from nucleotide monophosphates and glucosamine under the fermentative processes of baker's yeast. CDP-choline and CDP-aminoethanols were produced from CMP and choline and/or aminoethanols under the condition of yeast fermentation. 6-Phosphogluconate and galactose 1-phosphate were produced by coupling of baker's yeast and kinase systems. Respiration deficient mutants of yeast were employed to investigate the efficiency of energy generating system of yeast to produce CDP-choline and sugar nucleotides.

VIII. Studies on the Analytical Biochemistry[2,6,19,21,46,64,75]

New methods of spectrophotometric determination of keto acids, pyridoxal, pyridoxal phosphate, D-amino acids, and D-amino acid oxidase and ω-aminotransferase activity were established.

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