2.3.10 Laboratory : Biofunction Chemistry

Member :	Professor	Hideto Miyoshi, Dr. Agric. Sci.
	Assistant Professor	Masato Abe, Dr. Agric. Sci.
	Assistant Professor	Masatoshi Murai, Dr, Agric. Sci.
	Master's Program	12
	Undergraduate	4

A. Research Activities (2009.4-2010.3)

A-1. Main Subjects

a) Bioorganic chemical study for elucidating mitochondrial complex I

Proton-translocating NADH-ubiquinone oxidoreductase (complex I) is the initial enzyme of the mitochondrial respiratory chain. It couples the transfer of two electrons from NADH to ubiquinone to the translocation of four protons across the inner mitochondrial membrane. The enzyme is composed of 45 different subunits with a total molecular mass of about 1 MDa. Because of complexity of the enzyme, our knowledge about the molecular structure and the catalytic mechanism is still highly limited. The aim of our research is to get insights into the structural and functional features of complex I through syntheses of various molecular probes and studies on their inhibition mechanism.

On the basis of structure-activity studies on complex I inhibitors, we have designed and synthesized radioisotope-tagged photoaffinity labeling probes for identification of the binding sites in complex I. We have revealed that the ND1 subunit, which is located in the membrane domain, constructs the inhibitor binding domain and plays an important role in the energy coupling in complex I.

b) Synthesis of functional ubiquinone probes and its biochemical application

Ubiquinone is an important electron-carrier in the respiratory chain of most eukaryotic and prokaryotic organisms. Recently, another roles of ubiquinone in the cell, such as antioxidant and redox regulation, have been suggested. In order to elucidate overall function of ubiquinone in the cell as well as its function in the respiratory enzymes, we are synthesizing various biotinylated ubiquinone probes aimed at biochemical experiments such as affinity purification, Western analysis and uptake assay.

c) Construction of synthetic cardiolipin library

Cardiolipin, a phospholipid localized in the inner mitochondrial membrane, is believed to play important roles in the regulation of respiratory enzymes and the release of cytochrome c from mitochondria during the initial phase in apoptosis, while the details of the molecular mechanism is still unknown. We are constructing synthetic cardiolipin library, which can be used as powerful tools in various biochemical and biophysical experiments, to elucidate the biological functions in mitochondria.

A-2.Publications and presentations

a) Publications

Original Papers

- Mogi, T., Matsushita, K., Murase, Y., Kawahara, K., Hideto Miyoshi, H., Ui, H., Shiomi, K., Omura, S., and Kita, K. (2009) Identification of New Inhibitors for Alternative NADH Dehydrogenase (NDH-II), FEMS Microbiol. Lett. 291, 157-161
- Mogi, T., Ui, H., Shiomi, K., Ohmura, S., Miyoshi, H., and Kita, K. (2009) Antibiotics LL-Z1272 identified as novel inhibitors discriminating bacterial and mitochondrial quinol oxidases, Biochim. Biophys. Acta 1787, 129-133.
- Murai, M., Sekiguchi, K., Nishioka, T. and Miyoshi, H. (2009) Characterization of the Inhibitor Binding Site in Mitochondrial NADH-Ubiquinone Oxidoreductase by Photoaffinity Labeling Using a Quinazoline-Type Inhibitor, Biochemistry 48, 688-698.
- Kawahara, K., Mogi, T., Tanaka, T. Q., Masayuki Hata, Miyoshi, H., and Kita, K. (2009) Mitochondrial Dehydrogenases in the Aerobic Respiratory Chain of the Rodent Malaria Parasite Plasmodium yoelii yoelii, J. Biochem. 145, 229-237.
- Mogi, T. and Miyoshi, H. (2009) Properties of Cytochrome bd Plastoquinol Oxidase from the Cyanobacterium Synechocystis sp. PCC 6803, J. Biochem. 145, 395-401.
- Sekiguchi, K., Murai, M. and Miyoshi, H. (2009) Exploring the binding site of acetogenin in the ND1 subunit of bovine mitochondrial complex I, Biochim. Biophys. Acta 1787, 1106-1111.
- Mogi, T., Ano, Y., Nakatsuka, T., Toyama, H., Muroi, A., Miyoshi, H., Migita, C. T., Ui, H., Shiomi, K., Omura, S., Kita, K. and Matsushita, K. (2009) Biochemical and spectroscopic properties of cyanide-insensitive quinol oxidase from Gluconobacter oxydans, J. Biochem. 146, 263- 271.

b) Conference and seminar papers presented

- Annual Meeting of Japanese Society of Bioscience, Biotechnology, and Agrochemistry 2010: 2 report

- The 463th Kansai Branch Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry: 1 report
- The 24th Annual Meeting on Pesticide-Design: 1 report
- The 1st China-Japan-Korea Workshop on Pesticide Science: 1report
- The 11th International Kyoto Conference on New Aspect of Organic Chemistry (IKCOC-11): 1 report

A-3.Off-campus activities

Membership in academic societies

- H. Miyoshi, Dr. Agric, Sci. : Pesticide Science Society of Japan (councilor, editorial board member)

Research grants

1. Grants-in-aid for Scientific Research(KAKENHI)

- Scientific Research (B) : H. Miyoshi : Exploring the function of membrane domain of mitochondrial complex-I using inhibitor probes.
- Young Scientists (Start-Up) : M. Murai : Functional research on mitochondrial complex I based on the mode of action studies of the specific inhibitor.

A-4.International cooperation and overseas activities

International joint research, overseas research surveys

- Functional analysis of membrane domain subunits of mitochondrial complex-I through photoaffinity labeling study, H. Miyoshi, USA

B.Educational Activities(2009.4-2010.3)

B-1.On-campus teaching

a) Courses given

- Undergraduate level : ntroduction of applied life sciences IV (Miyoshi), Bioorganic chemistry II (Miyoshi), Laboratory course in bioorganic chemistry (Miyoshi, Abe, Murai)
- Graduate level : Chemistry of biologically active compounds (Miyoshi), Biofunction chemistry seminar (Miyoshi), Experimental course of biofunctional chemistry (Miyoshi).