2.3.3 Laboratory : Bioregulation Chemistry

Member :	Professor	Miyagawa, Hisashi, Dr. Agric. Sci.
	Associate Professor	Nakagawa, Yoshiaki, Dr. Agric. Sci.
	Assistant Professor	Miyashita, Masahiro, Dr. Agric. Sci.
	Doctor's program	1
	Master's Program	4
	Undergraduate	5
	Researcher	1

A. Research Activities (2009.4-2010.3)

A-1. Main Subjects

a) Structure-Activity Relationships of Ecdysone Agonists

The molting process is regulated by a molting hormone, 20-hydroxyecdysone (20E), in most invertebrates. To date 4 compounds which mimic the action of 20E are in the market as insecticide. The base structure of these compounds is dibenzoylhydrazine (DBH). DBHs are very toxic to Lepidoptera, but they are either very weak or inactive against other insect order. In this study, new structures are searched by in silico screening method. In the first step, 2.1 million structures are converted to 350 million 3D-structures. These 3-D stuctures are virtually screened to obtain 20,000 compounds based on shape and electrostatic similarity. These 20,000 structures were submitted to the 2nd screening to reduce the number of structures to 237 considering the QSAR results for ecdysteroids. Finally, 24 compounds were purchased and submitted to the bioassay to obtain three active compounds.

b) Chemistry of bioactive peptides in plants

Plants induce various defense responses when they are attacked by pathogens. These defense responses are triggered by a variety of molecules (elicitors). We previously discovered a novel small peptide, which can activate defense reactions in tobacco cells, from random hexapeptide libraries. In this study, in order to elucidate the mechanism of action of this peptide, signaling pathways involved in defense responses induced by the peptide was investigated. As a result, we found that this peptide activates the defense mechanism via the signaling pathway,in which jasmonic acid plays a important role.

c) Chemistry of scorpion toxins

We previously isolated two insecticidal toxins, LaIT1 and LaIT2, from the venom of the Japanese scorpion Liocheles australasiae. In this study, to investigate the structure-activity relationship of LaIT1, we synthesized several analogs of LaIT1 and examined its effects on insect toxicity. Substitution of each of two arginine residues, which is located at the central portion, to alanine resulted in a marked reduction of the activity, indicating that these residues are important for the interaction with the receptor.

d) Metabolism of Plant Hormone Auxin

Indole-3-acetic acid (IAA) is a plant hormone auxin that plays an important regulatory role in plant growth and development. Close examination of the metabolites in rice seedlings clarified that the formation of indole-3-carboxylic acid as well as hydroxyindole-3-carboxylic acid followed by the conjugation with glucose is one of the major pathways. Significant amounts of 3-hydroxy-2-oxo-IAA and its aspartate and glutamate conjugates were also detected. In the current year, we synthesized deuterated 3-hydroxy-2-oxo-IAA and its aspartate and glutamate conjugates to be used as internal standards for the quantification of their levels in plants.

A-2.Publications and presentations

a) Publications

Books

- Fujita, T. and Y. Nakagawa: SAR and QSAR Analyses of substituted dibenzoylhydrazines for their mode of Action as ecdysone agonists. Endocrine Disruption Modeling (Devillers, J., ed.), Taylor & Francis Group , Boca Raton, FL, pp359-379, 2009
- Nakagawa, Y., R. E. Hormann, G. Smagghe: SAR and QSAR studies for in vivo and in vitro activities of ecdysone agonists. Ecdysones: Structures and Functions (Smagghe, G., ed.), Springer, pp475-509, 2009
- Molecular mechanism for insect molting. Entomological Science and Its Perspective (Eds. Kenji Fujisaki, Ritsuo Nishida, and Masayuki Sakuma), Kyoto University Press, pp271-298, 2009
- Masahiro Miyashita and Yoshiaki Nakagawa. Study for toxins of Japanese scorpions, In “Entomological Science and Its Perspective (Eds. Kenji Fujisaki, Ritsuo Nishida, and Masayuki Sakuma”, Kyoto University Press, pp304-306, 2009

Original Papers

- Ishimoto, M., S. M. Rahman, M. S. Hanafy, M. M. Khalafalla, H. A. El-Shemy, Y. Nakamoto, Y. Kita, K. Takanashi, F. Matsuda, Y. Murano,T. Funabashi, H, Miyagawa

and K, Wakasa: Evaluation of amino acid content and nutritional quality of transgenic soybean seeds with high-level tryptophan accumulation. Molecular Breeding 25; 313-326, 2010
- Matsuda, F., A. Ishihara, K. Takanashi, K. Morino, H. Miyazawa, K. Wakasa and H. Miyagawa: Metabolic profiling analysis of genetically modified rice seedlings that overproduce tryptophan reveals the occurrence of its inter-tissue translocation. Plant Biotechnology 27; 17-27, 2010
- Soin, T., E. De Geyter, H. Mosallanejad, M. Iga, D. Martín, S. Ozaki, S. Kitsuda,T. Harada, H. Miyagawa, D. Stefanou, G. Kotzia, R. Efrose, V. Labropoulou, D. Geelen, K. Iatrou, Y. Nakagawa, C. R. Janssen, G. Smagghe, L. Swevers: Assessment of species specificity of moulting accelerating compounds in Lepidoptera: comparison of activity between Bombyx mori and Spodoptera littoralis by in vitro reporter and in vivo toxicity assays. Pest Manag Sci 66; 526-535, 2010
- Miyashita, M,. A. Sakai, N. Matsushita, Y. Hanai,Y. Nakagawa and H. Miyagawa: Novel amphipathic linear peptide with both insect toxicity and antimicrobial activity from the venom of the scorpion Isometrus maculates. Novel amphipathic linear peptide with both insect toxicity and antimicrobial activity from the venom of the scorpion Isometrus maculates. Biosci Biotech Biochem 74； 364-369, 2010
- Matsushita, N., M. Miyashita, Y. Ichiki, T. Ogura, E. Sakuradani, Y. Nakagawa, S. Shimizu and H. Miyagawa: Purification and cDNA cloning of LaIT2, a novel insecticidal toxin, from venom of the scorpion Liocheles australasiae. Biosci Biotech Biochem 73; 2769-2772, 2009
- Nakao, H., I. Matsunaga, D. Morita, T. Aboshi, T. Harada, Y. Nakagawa, N. Mori and M. Sugita: Mycolyltransferase from Mycobacterium leprae excludes mycolate-containing glycolipid substrates. J Biochem 146; 659-665, 2009
- Harada, T., Y. Nakagawa, M. Akamatsu and H. Miyagawa: Evaluation of hydrogen bonds of ecdysteroids in the ligand–receptor interactions using a protein modeling system. Bioorg. Med. Chem., 17; 5868-5873, 2009
- Beatty, J. M., G. Smagghe, T. Ogura, Y. Nakagawa, M. Spindler-Barth and V. C. Henrich: Properties of ecdysteroid receptors from diverse insect species in a heterologous cell culture system--a basis for screening novel insecticidal candidates. FEBS Journal 276; 3087-3098, 2009
- Harada, T., Y. Nakagawa, R. W. Wadkins, P. M. Potter and C. E. Wheelock: Comparison of benzil and trifluoromethyl ketone (TFK)-mediated carboxyesterase inhibition using classical and 3D-quantitative structure-activity relationship analysis. Bioorg Med Chem 17; 149-164, 2009

Reviews

- Nakagawa, Y. and V. C. Henrich: Arthropod nuclear receptors and their role in molting. FEBS J, 276; 6128-6157, 2009

- Miyagawa, H.: Studies on nitrogen-containing secondary metabolites playing a defensive role in plants. Nihonnouyakugakkaishi. 34: 127-135 (2009)

b) Conference and seminar papers presented

- Annual Meeting of the Japan Society for Bioscience, Biotechnology, and Agrochem istry 2009: 4 reports
- Annual Meeting of the Japan Society for Bioscience, Biotechnology, and Agrochem istry 2009: 4 reports
- The 56th Annual Conference on Mass Spectrometry: 1 report
- Japan Society for Bioscience, Biotechnology, and Agrochemistry (Kansai Branch Meeting): 1 report

A-3.Off-campus activities

Membership in academic societies

- Miyagawa, Hisashi Dr. Agric. Sci : Japan Society for Pesticide Science (councilor, editorial member), Japan Society for Bioscience, Biotechnology, and Agrochemistry (councilor of Kansai branch)
- Nakagawa, Yoshiaki Dr. Agric. Sci. : The Division of Structure-Activity Studies, The Pharmaceutical Society of Japan (board member), Japan Society for Pesticide Science (editorial board member, councilor), Japan Society for Bioscience, Biotechnology, and Agrochemistry (editorial board member), Japan Society for Bioscience, Biotechnology, and Agrochemistry-Kansai Branch (councilor)
- Miyashita, Masahiro; Dr. Agric. Sci. : The Mass Spectrometry Society of Japan (training planning committee member, journal editorial member)

Research grants

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

- Young Scientists (B) : Miyashita, Masahiro : Screening for plant defense activating peptides from combinatorial peptide Libraries

B.Educational Activities(2009.4-2010.3)

B-1.On-campus teaching

a) Courses given

- Undergraduate level: Bioorganic Chemistry I (Miyagawa, Nakagawa), Organic Reaction Mechanism II (Nakagawa), Laboratory Course in Bioorganic Chemistry (Miyagawa, Nakagawa, Miyashita), Structure Analysis of Organic Compounds (Miyagawa), Food Safety II (Miyagawa), Experimental Course in Division of Applied Life Sciences (Nakagawa, Miyashita); Pocket seminar (Miyagawa)
- Graduate level: Graduate level: Bioregulation Chemistry Seminar (Miyagawa, Nakagawa, Miyashita), Experimental Course in Bioregulation Chemistry (Miyagawa, Nakagawa, Miyashita).

B-2.Off-campus teaching etc.

Part-time lecturer

- Miyagawa, H.: School of Life and Environmental Sciences, Osaka Prefecture University (Molecular design), Faculty of Biotechnology, Fukui Prefectural University (Pesticide Chemistry), Tokyo University of Agriculture and Technology (Bioregulation chemistry)
- Nakagawa, Y.: Faculty of Agriculture, Kyoto Prefectural University (Industrial organic chemistry)

B-3.Overseas teaching

International students

- International students : Research Students 1 (USA)