DEVELOPMENTAL CHANGES OF GTP-CH I ACTIVITY IN INTEGUMENT AND FAT BODY OF THE SILKWORM, *BOMBYX MORI*

Tomomi Kato[1], Hiroshi Sawada[2], Takayuki Yamamoto[1], Keisuke Mase[3], Motoko Nakagoshi[1]
[1]Biological Laboratory, Center for Natural Sciences, Kitasato University, Sagamihara city, Kanagawa 228-8555, Japan, [2]Department of General Studies, Nihon University, Setagaya-ku, Tokyo 156-8550, Japan and [3]Department of Insect Biotechnology and Sericology, National Institute of Agrobiological Sciences, Matsumoto city, Nagano 390-0812, Japan

In insects, pteridines cause the body coloration, together with ommochromes and melanin. Among the pteridine derivatives, tetrahydrobiopterin (BH_4) is essential cofactor for the synthesis of neurotransmitters. In order to clarify the physiological roles of BH_4 in insects, we are investigating the biochemical properties of GTP-CH I, the first key enzyme in the biosynthesis of BH_4. We have reported strong signals of GTP-CH I mRNA were recognized around the periods of each ecdysis in the *quail*, whose characteristics were abundant accumulation of ommochromes and comparatively large larval markings formed by cuticular melanin. In this study, we examined GTP-CH I activity in the integument and the fat body at the various developmental stages. In the integument, GTP-CH I activity increased on the first day of the fifth-instar and gradually from late pupal stage to adult eclosion. However, the activity was extremely low around the period of larval-pupal ecdysis. These results indicate that BH_4 may have specific roles for the biosynthesis of both ommoclomes and melanin in the larval integument and the wings during the pigment formation.

ROLE OF TETRAHYDROBIOPTERIN ON PC12 CELLS PROLIFERATION

Kengo Fujimoto[1], Akemi Inaba[1], Setsuko Katoh[1,2], Akito Tomomura[1]
[1]Department of Biochemistry, Meikai University School of Dentistry, Sakado, Saitama 350-0283, Japan and [2]Laboratory of Biochemical Nutrition, Department of Food Science and Nutrition, Kyoritsu Women's University, Chiyoda-ku, Tokyo 101-8433, Japan

Sepiapterin reductase (SPR) is the important enzyme that catalyzes the last step in the biosynthetic pathway of tetrahydrobiopterin (BH_4). PC12 cells have been used as a model of catecholaminergic neurons in culture. For producing catecholamines, these cells require BH_4. In this study, we examined the effect of overexpression of SPR on cell proliferation of PC12 cells. SPR cDNA was cloned into expression vector. PC12 cells were seeded and then transfected using Lipofectamine with SPR expression vector. Both biopterin content and SPR activity in the SPR expression vector-transfected cells increased after the transfection. The cell did not show significant change in biopterin content, SPR activity, or growth rate after the transfection with control expression vector. These results indicated that transfection of PC12 cells with SPR expression vector for SPR increased their growth rate. Furthermore, we recognized that transcription factors for proliferation of cell were up regulated. Our data suggested that BH_4 content may control the proliferation of PC12 cells.

ACTIVATION SYSTEM OF ECDYSTEROID-PHOSPHATES DURING EARLY EMBRYONIC DEVELOPMENT IN THE SILKWORM *BOMBYX MORI*

Ryouichi Yamada[1], Kohei Atuta[1], Yumi Yamahama[2], Haruyuki Sonobe[1]
[1]Department of Life and Functional Material Science, Graduate School of Natural Sciences, Konan University, Kobe 658-8501, Japan and [2]Department of Biology, Hamamatsu University School of Medicine, Hamamatsu 431-3192, Japan

In various insect species, it has been accepted that ecdysteroid-phosphates are storage form for supplying active free ecdysteroids before the prothoracic grands of the embryo differentiate. We have demonstrated that ecdysteroid-phosphate phosphatase (EPPase), which specifically catalyzes the dephosphorylation of ecdysteroid-phosphates, participates in the increase of free ecdysteroids in *Bombyx* eggs. In this study, we indicated that EPPase is localized in the cytosol of yolk cells, and ecdysteroid-phosphates are bound to yolk protein vitellin (Vn) and stored in yolk granules. Furthermore, the Vn-bound ecdysteroid-phosphates were scarcely hydrolyzed by EPPase. To explain the process of hydrolysis of maternal ecdysteroid-phosphates by EPPase, we demonstrated that following biochemical reactions are operating: acidification of yolk granules, induced by vacuolar-type proton-translocating ATPase, triggers the dissociation of ecdysteroid-phosphates from Vn-ecdysteroid-phosphates complex and then ecdysteroid-phosphates are released to cytosol from yolk granules.

HIGH AFFINITY BINDING OF Zn ION AND ITS REACTION MECHANISM OF 20S PROTEASOME

Katuhiro Mishina[1], Shinpei Yamada[1,2]
[1]Department of Biology, Graduate School of Science and technology, Shizuoka University Shizuoka, Shizuoka 422-8529, Japan and [2]Department of Biology, Faculty of Science, Shizuoka University, Shizuoka, Shizuoka 422-8529, Japan

Many intracellular proteins are degraded by the proteasome system. Core of this system is 20S proteasome that has three peptidase activities (chymotrypsin, trypsin and caspase-like activities). We investigated that the effects of Zn ion and other divalent cation were analyzed on the three peptidase activities of 20S proteasome isolated from various animals and tissues. The results were obtained that three kinds of Zn ion binding were detected by measuring the peptidase activities of 20S proteasome and the mechanism were discussed.

ENZYMATIC PROPERTIES OF ECDYSTEROID KINASE IN OVARIES OF THE SILKWORM, *BOMBYX MORI*

Katsunori Ieki[1], Ryouichi Yamada[1], Yusuke Nagai[2], Haruyuki Sonobe[1,2]
[1]Department of Biology, Graduate School of Natural Science, Konan University, Kobe 658-8501, Japan and [2]Department of Biology, Faculty of Science and Engineering, Konan University, Kobe 658-8501, Japan

In ovaries of the silkworm, the bulk of ecdysteroids exists as phosphoric ester, such as ecdysone 22-phosphate, 20-hydroxyecdysone 22-phosphate, 22-deoxy-20-hydroxyecdysone 3-phosphate and 2,22-dideoxy-20-hydroxyecdysone 3-phosphate. However, little or no attention has been paid to ecdysteroid kinase involved in the synthesis of the phosphoric ester in *Bombyx* ovaries. In this study, ecdysteroid kinase activity was measured using ^{3}H-ecdysone as the substrate. Radioactivity of ^{3}H-ecdysone 22-phosphate converted from ^{3}H-ecdysone was quantified using a liquid scintillation counter. We attempted partial purification of ecdysteroid kinase in *Bombyx* ovaries, and some enzymatic properties were demonstrated using the partially purified enzyme. It is worthy of our notice that ecdysteroid kinase, partially purified in our present experiments, catalyzed the phosphorylation of ecdysone and 20-hydroxyecdysone at the C-22 position, but did not catalyze the phosphoryaltion of 22-deoxy-20-hydroxyecdysone and 2,22-dideoxy-20-hydroxyecdysone at the C-3 position. These results suggest that the phosphorylation of ecdysteroids at C-22 and C-3 positions are catalyzed by different enzymes.

ANALYSIS OF *DAPHNIA* RESTING EGGS BY ELECTRON SPIN RESONANCE (ESR) SPECTROSCOPY

Masanobu Sakata[1], Tamami Kawasaki[1], Toshimichi Shibue[2], Atsushi Takada[1], Hideyuki Yoshimura[3], Hideo Namiki[1]
[1]Department of Integrative Bioscience and Biomedical Engineering, Graduate School of Science and Engineering, Waseda University, Shinjyuku-ku, Tokyo 169-8555, Japan, [2]Materials Characterization Central Laboratory, Waseda University, Shinjyuku-ku, Tokyo 169-8555, Japan and [3]Department of Physics, Meiji University, Kawasaki-shi, Kanagawa 214-8571, Japan

Daphnia normally reproduce by parthenogenesis. In unfavourable environments, however, robust resting eggs are induced after they switch to sexual reproduction. Resting eggs can remain viable for decades or centuries, and can withstand freezing and drying. Resting eggs are also able to survive in the harsh environment of a predator's digestive system. However, there is a little physico-chemical information about the resting eggs itself. A previous study reported the existence of magnetic material having properties consistent with magnetite in *Daphnia* resting eggs (Zool.Sci.(2004)21:p.63-67). In this study, the metal that gives the magnetic property to *Daphnia* resting eggs was investigated by Electron Spin Resonance (ESR) spectroscopy. The ESR spectroscopy is used to detect presence of unpaired spins from metal elements. As a result, two ESR signals were obtained from *Daphnia* resting eggs. One ESR signal was matched to the ESR signal originated from Fe^{3+}, and another ESR signal was matched to the ESR signal originated from magnetite.

INVOLVEMENT OF GROWTH FACTORS AND MUSCLE SATELLITE CELLS IN CLENBUTEROL-INDUCED HYPERTROPHY OF RAT MASSETER MUSCLE

Satonari Akutsu[1], Akira Yamane[2]
[1]High-Technology Research Center, Tsurumi University School of Dental Medicine, Tsurumi-ku, Yokohama, Kanagawa 230-8501, Japan and [2]Department of Pharmacology, Tsurumi University School of Dental Medicine, Tsurumi-ku, Yokohama, Kanagawa 230-8501, Japan

Recently, we have reported that clenbuterol, a β_2-adrenergic agonist, induces the hypertrophy of rat masseter muscle. The purpose of the present study is to determine if growth factors and muscle satellite cells are involved in the clenbuterol-induced hypertrophy of rat masseter muscle. Clenbuterol (30 μg/ml) was orally administered to 8 week-old rats via their drinking water for 3 weeks. The mRNA expression levels for growth factors (TGFβ, FGF, PDGF, HGF and myostatin) and for satellite cell markers (pax7, m-cadherin, MNF, myoD and myogenin) were analyzed by competitive-polymerase chain reaction in combination with reverse-transcription. Clenbuterol induced approximately 50 - 110% increases in the mRNA expression levels for TGFβ1-3, PDGF-B and myostatin, but it induced an approximately 30% decrease in the mRNA expression level for FGF-2. Clenbuterol induced approximately 40% increases in the mRNA expression levels for m-cadherin, MNF, myoD and myogenin. These results suggest that those growth factors and myogenic satellite cells are involved in the clenbuterol-induced hypertrophy of rat masseter muscle.