THE ROLE OF PIGMENT BINDING PROTEIN IN XANTHOMMATIN BIOSYNTHESIS IN THE EPIDERMAL CELLS OF THE SILKWORM, *BOMBYX MORI*.

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We have already reported that the pigment protein separated by SDS-PAGE had an activity binding with xanthommatin and cinnabarinic acid. However, 3-hydroxy-kynurenine or 3-hydroxy-anthranilic acid did not bind to the protein. These data indicate that the protein has an affinity with phenoxazinone ring of xanthommatin. Biosynthesis of xanthommatin was also investigated. The homogenate of epidermal tissue was fractionated by sucrose density gradient centrifugation. When the substrate 3-hydroxy-kynurenine was added to each fraction, catalytic activity to form xanthommatin localized in the pigment granules fraction. In addition, HPLC analysis revealed the incorporation of 3-hydroxy-kynurenine into the intact pigment granules. These data suggest that the pigment granule has an important role in both biosynthesis and accumulation of xanthommatin.

Recently, we obtained polyclonal antibody from the pigment protein which was purified by SDS-PAGE. Furthermore, we are now investigating the cross-reactivity of the antibody with crude extract of epidermis of *w2* mutant which is unable to synthesize the pigment.

PTERIDINES IN THE YELLOW-COLORED CHROMATOPHORES OF THE ISOPOD, *ARMADILLIDIUM VULGARE*.

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In *A. vulgare* the ommochromes in the integument impart a dark gray or brown body color. The male of *A. vulgare* displays a uniform dark gray color, while the female usually displays a brown color with characteristic yellow markings aligned along the dorsal region. Principal component of the yellow pigment isolated from the yellow markings was already identified as sepiapterin. Morphological investigations revealed that the pattern of yellow-colored chromatophores in the female was externally observable at the dorsal surface of the integument as the yellow markings. In contrast, the yellow-colored chromatophores were not externally observable in the male, since they were covered by an ommochrome chromatophore layer. The yellow-colored chromatophores contained numerous granules in the cytoplasm and the morphological properties of the granules were similar to those of pteridine granules which contain uric acid occurring in the silkworm integument. Based on TLC, HPLC and UV-spectrophotometric analyses, we concluded that blue and violet fluorescent compounds isolated from the chromatophores were biopterin, pterin and isoxanthopterin. Uric acid also accumulated in the chromatophores. The content of both sepiapterin and biopterin in the male was about two times greater than in the female, while the content of both pterin and isoxanthopterin showed few difference between the male and female. The quantitative difference in sepiapterin and biopterin between both sexes suggests that the activities of various enzymes involved in pteridine metabolism may differ between the male and female of *A. vulgare*.

CHARACTERIZATION OF RAT AND HUMAN SEPIAPTERIN REDUCTASE GENES

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Biologically active biopterins observed in insects and vertebrates are produced by the function of sepiapterin reductase (SPR). We studied on the characterization of rat and human genes of SPR. We have isolated a full-length cDNA clone for SPR from a human liver cDNA library by plaque hybridization and analyzed the nucleotide sequence of the cDNA[1]. We amplified the cDNA for rat SPR by the PCR.Synthetic primers for the amplification were designed based on the nucleotide sequence of rat SPR[2] and the amino acid sequence of the mature form of rat SPR[3]. The clone encoded a protein of 261 amino acids (783 bases) with a calculated Mr of 28047 daltons.A single gap of codon was introduced into the human SPR sequence against rat SPR [1*AGG]. Consensus sequences for NADPH and pterin located in the range near the 5'-end in both SPR. Pterin binding site [GCCGGGTTG-CTGTCG(A-G-L-L-S)] for rat SPR[2] was revealed as [GCCTCGCTGCTGTCG(A-S-L-L-S)] in human SPR. Estimation of the number of nucleotide substitution was 0.250± 0.021(total;JC method), and 0.262, 0.105, and 0.429 at the 1,2, & 3 base positions of codon, respectively (K3P method). Human SPR showed a 74% identity in amino acid sequence with that of rat SPR. Rate of amino acid substitution was 1.9×10^{-9}.
[1]Ichinose et al'91,BBRC179,183;[2]Citron et al '90,PNAS87,6436;[3]Oyama et al'90,BBRC173,627.

THE STUDY ON BODY COLOR OF ARMADILLIDIUM VULGARE BY THE FINE STRUCTURE OF EPIDERMIS AND PIGMENT QUANTITY.

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A.vulgare is polymorphic for the body color. Red body color is dominant over the usual black or grey (wild type). Polymorphism may be provided by the difference in the fine structure of pigment granules and/or pigment quantity under the control of proper genes. The study was undertaken to prove this hypothesis.

Chromatophores of the red phenotype were filled with pigment granules of filamentous structures such as immature granules occurring within the same limiting membrane. Xanthommatin content of red A.vulgare is much the same with that of wild type. Epidermis of white A.vulgare is very partially pigmented in the large vesicles. This observation suggests that white woodlice provide the enzyme for ommochrome synthesis and its activity may be prohibited though the mechanism is uncertain. These results show that the structure of pigment granules is determined by the corresponding gene to the body color.