작잠번데기 체액 주단백질의 생리생화학 및 분자생물학적 특성

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A pupal major haemolymph protein of the Chinese wild oak silkmoth *Antheraea pernyi* (AMHP) was purified, identified and characterized physiologically, biochemically, immunologically and molecular biologically. The protein AMHP was purified by a simple preparative polyacrylamide gel electrophoresis ((PAGE) and diffusive elution. The preparation was shown to be homogeneous by 7.5% native-PAGE. The native molecular weight of the AMHP was 450kDa with a 80kDa single subunit, suggesting hexamer. The protein contained high amounts (18.3%) of aromatic amino acids, e.g. phenylalanine(9.7%) and tyrosine(8.6%). Therefore, the protein was identified as a kind of a storage protein referred to as an arylphorin (aromatic amino acid-rich storage protein). The protein was stained by Schiff's reagent, suggesting a glycoprotein. The protein contained 4.9%(w/w) sugar, and mannose and N-acetylglucosamine were major components. Also, degradation of protein was begun by heat treatment at 90℃ for 20 minutes. From these results, it was concluded that the AMHP is a storage protein referred to as arylphorin which is purified from the pupal haemolymph of *A. pernyi*.

Immunological properties and the stage-dependent synthesis of the AMHP of *A. pernyi* were also investigated. Anti-AMHP serum was prepared by immunizing the mouse with AMHP purified from the pupal haemolymph of *A. pernyi*. In Western blot analysis following native- and SDS-PAGE, the antibody against AMHP cross-reacted with the AMHP in the haemolymph of both the sexes excluding the male adult during all the developmental stages from egg to adult. Also, the antibody cross-reacted with the crude protein extract of the haemolymph, integument, fat body, midgut, excluding silk gland, in the 5th instar larvae. By *in vivo* tracer experiments using [^{14}C]-leucine, the synthetic activity was detected strongly in the haemolymph of the early larvae, weakly in the haemolymph of the

middle and late larvae in the 5th instar, but absolutely not in the haemolymph of the pupal stage. Also, in *in vitro* synthesis analysis of the 5th larval fat body using [^{14}C]-leucine tracer the larval fat body was proved to be a site for the synthesis of the AMHP. These results revealed that the AMHP is synthesized strongly in the 5th larval fat body, and the synthesis can be ceased befor larval-pupal transformation.

The *A. pernyi* AMHP is characterized as a hexameric haemolymph protein with 80kDa single subunit. The cDNA and the developmental profiles of the mRNA for *A. pernyi* AMHP has been determined. The complete *A. pernyi* AMHP cDNA sequence comprised 2,234bp (without the poly A^+ tail), including an open reading frame of 2,112bp beginning with a methionine ATG at bp 34. The *A. pernyi* AMHP contained 704 amino acids which are highly enriched in aromatic amino acids, phenylalanine and tyrosine. The calculated molecular mass of the *A. pernyi* AMHP from the ORF was 83,439 dalton. The deduced amino acid sequence of *A. pernyi* AMHP showed 78, 71, 62 and 64% identity with those of *H. cecropia, M. sexta* α subunit, *M. sexta* β subunit and *B. mori* storage protein. In Northern blot analysis, the *A. pernyi* AMHP mRNA only in the fat body of the 5th instar larvae was responsible for the gene expression of the protein, and the synthetic activity of the mRNA was detected strongly in the early larvae, but not in the middle or late larvae. In addition, very weak signal in mRNA activity was detected in pupal stages, but this is considered as inactive mRNA by viewing the results of this protein labelling experiment in the present study.