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A cDNA encoding diazepam-binding inhibitor/acyl-CoA-binding protein in *Helicoverpa armigera*: Molecular characterization and expression analysis associated with pupal diapause

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Abstract

The diazepam binding inhibitor (DBI) or the acyl-CoA-binding protein (ACBP) is a 9–10 kDa highly conserved multifunctional protein that plays important roles in $GABA_A$ receptor activity regulation, lipid absorption and steroidogenesis in various organisms. To study the functions of DBI/ACBP in insect development or diapause, we cloned the cDNA from *Helicoverpa armigera* (Har) utilizing rapid amplification of cDNA ends (RACE). By homology search, Har-DBI/ACBP is conserved with the DBI/ACBPs known from other insects. Northern blot analysis showed that DBI/ACBP gene expressed in nonneural and neural tissues. RT-PCR combined Southern blot analysis revealed that DBI/ACBP mRNA in the brain of nondiapause individual was much higher than that in the brain of diapausing insects. At early and middle stages of 6th instar larvae, the level of DBI/ACBP mRNA was higher in the midgut of diapause type than that in nondiapause type and low at late 6th instar larval stage and early pupal stage in both types. In the prothoracic gland (PG), DBI/ACBP expression appeared at a high level at middle and late stages of 6th larval instar in both nondiapause and diapause types, and declined after pupation. In vitro experiments revealed that DBI/ACBP mRNA in PG could be stimulated by synthetic *H. armigera* diapause hormone (Har-DH), suggesting that Har-DH may stimulate the PG to produce ecdysteroids by the DBI/ACBP signal pathway. By in vitro assay, we also found that FGIN-1-27, which has similar functions to DBI/ACBP in ecdysteroidogenesis, could induce PG ecdysteroidogenesis effectively, suggesting that DBI/ACBP regulates biosynthesis of ecdysteroids in PG. Thus, DBI/ACBP indeed plays a key role in metabolism and development in *H. armigera*.

Keywords: Diazepam-binding inhibitor/acyl-CoA-binding protein; cDNA cloning; Gene expression; Diapause; In situ hybridization; Prothoracic gland; Ecdysteroidogenesis; *Helicoverpa armigera*

1. Introduction

An endogenous protein, which is initially discovered in mammalian brain with the ability to displace benzodiazopine analogs from $GABA_A$ receptors, is named as diazepam-binding inhibitor (DBI) (Guidotti et al., 1983). Subsequently, DBI is also found in non-vertebrates such as yeast, insects and plants (Rose et al., 1992; Synder and Fevereisen, 1993; Kolmer et al., 1994; Newman et al., 1994). In addition to the previously mentioned nervous system function as a regulator of $GABA_A$ receptor activity, DBI protein possesses at least two other major functions: 1) DBI can specifically bind medium to long-chain acyl-CoA fatty acid esters, so that it is also called the acyl-CoA-binding protein (ACBP) (Mikkelsen et al., 1987; Rosendal et al., 1993; McClelland, 2004); 2) DBI/ACBP can promote steroidogenesis via binding to a specific outer mitochondrial membrane receptor (Papadopoulos et al., 1991; Krueger and Papadopoulos, 1992). Most DBI/ACBP proteins are 9–10 kDa in molecular mass, 86–104 residues. DBI/ACBP protein sequences share more than 50% homology at amino acid level in most insects. The

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highly conserved sequence between phylogenetic groups is likely to indicate common functions for DBI/ACBP in insect species.

Insect molting, metamorphosis and diapause are modulated by a class of steroid hormones, especially 20-hydroxyecdysone (Denlinger, 2002; Gilbert et al., 2002). The prothoracic gland (PG) is stimulated to produce ecdysteroids by prothoracicotropic hormone (PTTH), which is secreted from the brain (Gilbert et al., 2002). Pupal diapause is usually caused by a decrease in the hemolymph ecdysteroid titer, due to a decrease in the biosynthetic activity of the brain, which produces PTTH (Denlinger, 1985, 2002). Diapause pupae can be stimulated to initiate development at any time by a simple injection of the ecdysteroid, 20-hydroxyecdysone (Denlinger, 1985). Recently, we found that diapause hormone (DH), a neuropeptide produced in the suboesophageal ganglion (SG), could also break pupal diapause efficiently in *Helicoverpa armigera* through stimulating PGs to synthesize the ecdysteroids (Zhang et al., 2004a,b). Yet, the mechanism of DH regulating steroidogenesis is unknown.

DH breaks pupal diapause through elevating the ecdysteroid titer in hemolymph (Zhang et al., 2004b), and DBI/ACBP plays an important role in steroidogenesis (Papadopoulos et al., 1991; Krueger and Papadopoulos, 1992). Therefore, we focus on the possible function of DBI/ACBP in *H. armigera* pupal diapause.

In the present paper, we isolate a cDNA encoding the DBI/ACBP peptide from the PG, and investigate its expression patterns. The results suggest that the DBI/ACBP is involved in the regulation of ecdysteroid biosynthesis, larval and pupal development in *H. armigera*.

2. Materials and methods

2.1. Insect

Larvae of cotton bollworm (*H. armigera*) were reared on an artificial diet at 25±1 °C and L14:D10 (light:dark) photoperiod to generate nondiapause pupae, and at 18±1 °C with a cycle of L10:D14 to generate diapause pupae. Thus, the duration of 6th instar of nondiapause-destined individuals would be 6±1 days, and that of diapause-destined individuals would be 12±1 days. The development stages were synchronized at each molt by collecting new larvae or pupae. Brain, midgut and prothoracic glands were dissected in 0.75% NaCl and stored at −70 °C.

2.2. Total RNA extraction, cDNA synthesis and PCR amplification

Total RNA was prepared from PGs of 6th instar larvae by the single-step method of acid guanidinium thiocyanate-phenolchloroform extraction according to Chomczynski and Sacchi (1987). The first strand cDNA was synthesized from 1 μg of total RNA at 42 °C for 1 h with reverse transcriptase AMV (TaKaRa Co., Dalian, China). The degenerate primers DP1 (5′-CAATT(C/T)(G/A)ACCA(A/G/C)GCCGC-3′) and DP2 (5′-TCCAC(C/G/A)(A/T)T(C/G/T)(T/G)CGATGTA-3′) (Fig. 1) were synthesized by TaKaRa based on known DBI/ACBP cDNAs (Synder and Fevereisen, 1993; Kolmer et al., 1994; Matsumoto et al., 2001). PCR amplification was performed using the degenerate primers under the following conditions: after 5 min at 93 °C, 30 cycles of 60 s at 93 °C, 60 s at 50 °C, 60 s at 72 °C, then 10 min at 72 °C.

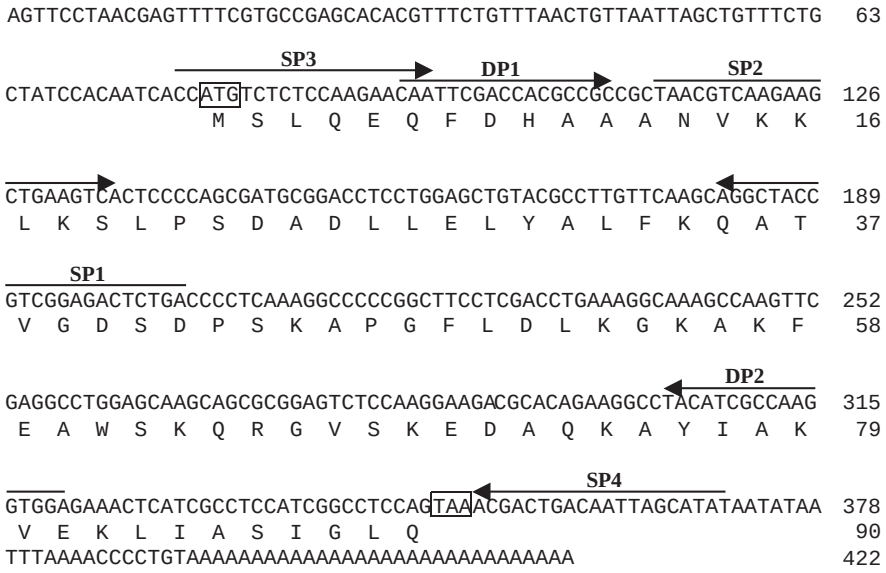

Fig. 1. Nucleotide and deduced amino acid sequences of Har-DBI/ACBP cDNA. The suggested start ATG and the stop codon TAA are indicated in boxes. Arrows above the nucleotide sequence represent the position of the different synthetic primers used in PCR. Degenerate primers are DP1 and DP2. Specific primers for RACE and PCR are SP1, SP2, SP3 and SP4.