

Identification of Dopa decarboxylase associated protein from *Bombyx mori*

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Objectives

Current study was aimed to understand an interaction between Dopa decarboxylase (DDC) and proteins that specifically binds to DDC in the silkworm, *Bombyx mori*

Materials and Methods

Materials - Animal : *Bombyx mori*

Construction of GST fusion protein

Preparation of lysates : Protein extracted from whole body of *Bombyx mori*

Methods - In vitro binding assay with lysates, Peptide sequence and RACE-PCR

Results and Discussion

In this study, we prepared the glutathione S-transferase (GST)-DDC fusion protein in *E. coli* and incubated it with silkworm tissue extracts. As a result, we found a 20 kDa protein that specifically interacts with GST-DDC. A cDNA fragment of dopa decarboxylase(DDC) binding 20 kDa protein from fat-body fo *Bombyx mori* was amplified by 3'RACE-PCR using degenerate primers based on the N-terminal amino acid sequence of purified 20 kDa protein. Amino-terminal peptide sequence followed by 3'RACE-PCR revealed that *Bombyx mori* has a 600 bp gene encoding a protein that specifically binds to DDC. We are currently identifying the gene with DNA sequencing.

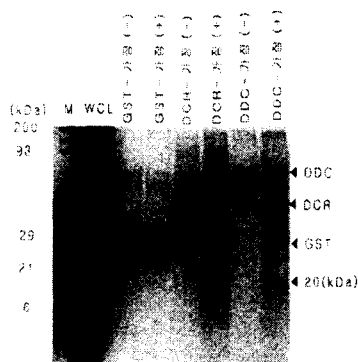


Fig. 1. 20 kDa protein specifically binds to DDC

1. PKVVFTYFSTKALGESGRMMLXYY,
2. PKVVFTYFSTKALGESGRMMLCYY,
3. PKVVFTYFSTKALGESGRMLLXYY,
4. PKVVFTYFSTKALGESGRMLLCYY.

Fig. 2. N-terminal peptide sequences from purified 20 kDa protein

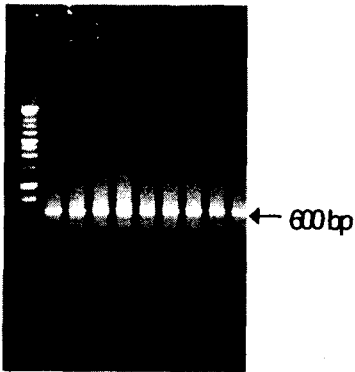


Fig.3. 3'RACE-PCR products of purified 20 kDa protein

References

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2. Dopamine is a key factor for the induction of egg diapause of the silkworm, *Bombyx mori*. Noguchi, H., Hayakawa, Y., (2001), *Eur. J. Biochem.*, 268: 774-780.