

**Molecular cloning and characterization of the dopa decarboxylase gene from
*Antheraea yamamai***

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Objectives

To understand the relationship between DDC activity and diapause or other biological events in *Antheraea yamamai*, we have determined a complete cDNA sequence of DDC homologue from *Antheraea yamamai* and have examined expression patterns in various tissues by Northern blot analysis.

Materials and Methods

Materials - Animal : *Antheraea yamamai* (Japanese oak silkworm)

Preparation of mRNA : Poly(A)+RNA was isolated from fat body

Methods - RT-PCR, Northern blot analysis, sequence data analysis : DNASTAR

Results and Discussion

Antheraea yamamai DDC cDNA was generated by RT-PCR as described in Materials and methods. The full cDNA sequence of *Antheraea yamamai* DDC contains 1,437bp encoding a protein of 478 amino acids. DDC contains one molecule of pyridoxal 5-phosphate (PLP) per enzyme dimer and PLP binding to DDC is critical for enzyme catalysis (Voltattorni *et al.* 1979). The putative PLP-binding site and its adjacent sequence (NFNPHKW) of *Antheraea yamamai* DDC are well conserved among insects species. The calculated molecular mass of the *Antheraea yamamai* DDC based on the DNA sequence is 54,662 Da (pI=5.73). When the deduced amino acid sequence of the *Antheraea yamamai* DDC was aligned with those of *Manduca sexta* (GenBank accession U03909), *Drosophila melanogaster* (GenBank accession P05031), *Ceratitis capitata* (GenBank accession Y11906) and *Aedes aegypti* (GenBank accession U27581) *Bombyx mori* (GenBank accession AF372836), the amino acid identity was 87%, 73%, 68%, 72% and 98%, respectively. And also we analyzed the phylogenetic relationship of entire amino acid sequences among Ay-DDC and other DDC family members.

To examine tissue expression of *Antheraea yamamai* DDC mRNA, we performed Northern blot analysis with total RNAs isolated from epidermis, silk gland, midgut and fat-body from final instar larvae.

Reference

Voltattorni CB, Minelli A, Vecchini P, Fiori A, Turano CM (1979) Purification and characterization of 3,4-dihydroxyphenylalanine decarboxyase from pig kidney. *Eur. J. Biochem.* **93**: 181-188.