

甜菜夜蛾触角结合蛋白 II 的 cDNA 克隆、组织分布及配体结合特性分析

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cDNA cloning, tissue distribution and ligand binding characteristics of antennal binding protein 2 from the beet armyworm, *Spodoptera exigua* (Lepidoptera: Noctuidae)

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摘要 触角结合蛋白 (antennal binding proteins, ABPs) 是气味结合蛋白 (odorant binding proteins, OBPs) 的一个亚类, 推测其在昆虫嗅觉中起作用。为了探讨这一问题, 本研究通过转录组数据分析并利用 RACE 技术, 克隆了甜菜夜蛾 *Spodoptera exigua* 触角结合蛋白 II 基因 (*SexigABP2*) 的全长 cDNA 序列 (GenBank 登录号为 HQ234486)。序列分析表明, 该基因开放阅读框长 444 bp, 编码 148 个氨基酸, 具有 OBPs 典型的 6 个半胱氨酸位点; 其氨基酸序列和烟芽夜蛾 *Heliothis virescens* 的 HvirABP2 的一致性最高, 达 72%。实时定量 PCR 分析显示, 该基因主要在触角中表达, 在喙、足、翅等组织中也有少量表达, 且在雌蛾触角及足中的表达量显著高于雄蛾。进一步对该基因进行原核表达和纯化, 利用荧光竞争结合实验测定了 *SexigABP2* 对 35 种气味物质的结合能力, 发现其对甜菜夜蛾性信息素组分 (*Z*)-9-十四碳烯醇和植物挥发物法尼醇的结合能力较强, 结合常数分别为 8.24 $\mu\text{mol/L}$ 和 8.14 $\mu\text{mol/L}$ 。结合能力比较表明, *SexigABP2* 对不饱和和长碳链化合物较饱和和短碳链化合物具有更强的结合能力; 在不饱和和长碳链化合物中, 对醇类物质又较乙酸酯类物质具有更强的结合能力。结果提示 *SexigABP2* 可能参与了成虫对不饱和和长碳链的植物挥发物的感受。

关键词: 甜菜夜蛾 触角结合蛋白 基因克隆 组织表达谱 配体结合 荧光竞争结合实验

Abstract: Antennal binding proteins (ABPs) represent a sub-class of odorant binding proteins (OBPs), and thus are assumed to play a role in insect olfaction. In order to explore the role of ABPs in olfaction, the full-length cDNA of an antennal binding protein 2 gene from *Spodoptera exigua* (*SexigABP2*) (GenBank accession no. HQ234486) was identified by transcriptome analysis and RACE technology. The sequence analysis showed that *SexigABP2* contains a 444 bp open reading frame that encodes 148 amino acids including the six conserved cysteine residues of typical OBPs. *SexigABP2* shares the highest amino acid identity (up to 72%) with an ABP2 from *Heliothis virescens* (HvirABP2). The results of real-time quantitative PCR showed that *SexigABP2* was highly expressed in male and female antennae, but weakly expressed in proboscis, legs, and wings of both sexes. The expression levels in female antennae and legs were significantly higher than those in male antennae and legs, respectively. *SexigABP2* was further expressed in a prokaryotic expression system, and the protein was purified. By fluorescence competitive binding assay, the affinities of *SexigABP2* with 35 odorant compounds were tested. Among the tested ligands, (*Z*)-9-tetradecenol (a sex pheromone component of *S. exigua*) and farnesol (a plant volatile compound) showed the highest affinity, with the K_i values of 8.24 $\mu\text{mol/L}$ and 8.14 $\mu\text{mol/L}$, respectively. Affinity comparison indicated that long carbon-chain compounds with unsaturated bond(s) exhibited the higher affinities than short ones without unsaturated bond; among the unsaturated long carbon-chain compounds, however, alcohols displayed higher affinities than acetates. The results suggest that *SexigABP2* might be involved in perception of plant volatile compounds with a long carbon-chain and unsaturated bonds.

Key words: *Spodoptera exigua* antennal binding protein gene cloning tissue expression pattern ligand binding fluorescence competitive binding assay

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