Journal of Chemical Ecology, Vol. 28, No. 4, April 2002 (© 2002)

Originally published online February 14, 2002,
Rapid Communications, pp. RC1–RC6
(http://www.kluweronline.com/issn/0098-0331)

IDENTIFICATION AND CLONING OF A FEMALE ANTENNA-SPECIFIC ODORANT-BINDING PROTEIN IN THE MOSQUITO *Culex quinquefasciatus*

YUKO ISHIDA,[1] ANTHONY J. CORNEL,[2] and WALTER S. LEAL[1,*]

[1]*Honorary Maeda-Duffey Laboratory*

[2]*Mosquito Control Research Laboratory, Department of Entomology, University of California Davis, Davis, CA 95616*

(Received January 16, 2002; accepted February 5, 2002)

Abstract—We have identified and cloned a cDNA encoding the first odorant-binding protein isolated from mosquitoes. The protein isolated from female antennae of *Culex quinquefasciatus* (CquiOBP) was not detected in legs (control tissue) or in antennal extracts from males, and showed mobility in native polyacrylamide gels similar to that of the pheromone-binding protein from *Bombyx mori.* The open reading frame of the cloned cDNA encoded a hydrophobic signal peptide (24 residues) and an acid mature protein (pI 5.5) of 125 amino acid residues (calculated molecular mass 14,504 Da). The transcript was detected by RT-PCR with antennal, but not with leg tissues. CquiOPB shared the highest amino acid identity with a product deduced from *Drosophila melanogaster* PBPRP-3 cDNA (58.6%), OBPs from scarab beetles (35%), and moths (28%). In addition, CquiOBP showed the hallmark of insect odorant-binding proteins, the six Cys residues.

Key Words—Odorant-binding protein, mosquito, *Culex quinquefasciatus*, Diptera, Culicidae.

INTRODUCTION

Female mosquitoes perceive the environment through semiochemicals, which they utilize for essential tasks, such as location of hosts (Davis and Bowen, 1994) and oviposition sites (Laurence and Pickett, 1982). To guide the mosquito behavior, these molecules must reach the olfactory receptors located in the dendrites, which are surrounded by a sensillar lymph. The transport of the hydrophobic semiochemicals through this aqueous environment is assisted by proteins, odorant-biding

* To whom correspondence should be addressed. E-mail: wsleal@ucdavis.edu

proteins (OBPs), which solubilize the ligands, ferry them to their receptors, and protect the molecules from inactivation before their messages are delivered. Research has been conducted to gain a better understanding of the molecular basis of semiochemical perception in the cosmotropical nuisance mosquito *Culex quinquefasciatus* Say (Diptera: Culicidae). *Cx. quinquefasciatus* is a vector of bancroftian filariasis caused by the nematode *Wuchereria bancrofti*, a debilitating helminthic disease that afflicts 119 million people world-wide (Michael et al., 1996). Here, we report the first isolation, identification, and cDNA cloning of a dipteran OBP.

METHODS AND MATERIALS

Protein Extracts and Analytical Procedures. Three to five-day old adult mosquitoes were anesthetized on ice. Females were inseminated, but deprived of a blood meal. Antennae and control tissues (midlegs with tarsi removed) were dissected and homogenized in ice-cold glass homogenizers with 10 mM Tris-HCl, pH 8. Homogenized samples were centrifuged twice at 12,000 rpm for 10 min at 4°C. After concentration, the supernatants were analyzed by native polyacrylamide gel electrophoresis (15% PAGE) and stained with Coomassie blue R-250. For sequencing, proteins were electroblotted onto polyvinyl difluoride membranes, bands were cut, and N-terminal sequences were obtained on a Procise Protein Sequencing System (Applied Biosystems).

cDNA Cloning and RT-PCR. mRNA samples were prepared from 1,000 antennae and 200 midlegs of female *Cx. quinquefasciatus* using TRIzol (Invitrogen) and PolyATract mRNA Isolation Systems (Promega). cDNAs were synthesized by SMART RACE cDNA Amplification Kit (Clontech). *TAKARA LA Taq* (TAKARA) was used as Taq polymerase. To clone CquiOBP cDNA, we used the following primers: the degenerate primer 5′-GA(C/T)GC(A/C/G/T)GA(A/G)TA(C/T)-CC(A/C/G/T)CC(A/C/G/T)CC(A/C/G/T)GA-3′ was designed on the basis of N-terminal amino acid sequence of the isolated protein, GCGTTTTTTTTTTTTTT (3′-RACE), a UPM primer from the SMART RACE cDNA Amplification Kit and a gene-specific primer (GSP1; 5′-RACE), 5′-CCCCCTCCGGATACAGGCAACG-TTTTCCCA-3′, and GSP-2 (RT-PCR), 5′-CGAGGCCATCATTGAGTTCAGC-GACGGCAA-3′. Polymerase chain reactions (PCR) were carried out according to instruction manual of the SMART RACE cDNA Amplification Kit. PCR products were ligated into pBluescript SK(+) (Stratagene) and sequenced. Homology analyses were carried out using FASTA (http://www.ddbj.nig.ac.jp/). Alignment of amino acid sequences was drawn by MacVector (Oxford Scientific).

RESULTS AND DISCUSSION

Native polyacrylamide gel electrophoresis analysis of protein extracts from antennae and legs of *Cx. quinquesfasciatus* showed the occurrence of an

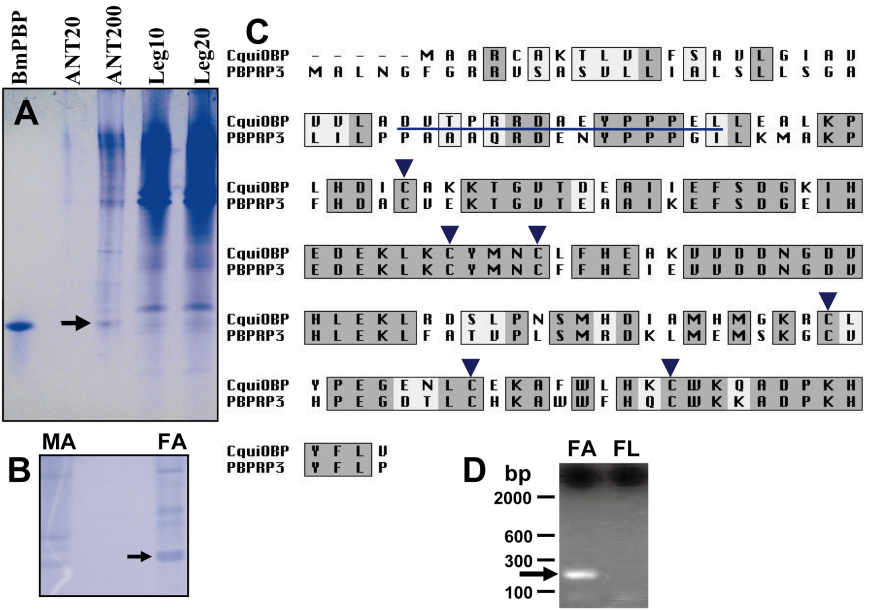

FIG. 1. Gel electrophoresis analysis and cDNA cloning data of the female- and antenna-specific protein from *Cx. quinquefasciatus.* A) Soluble proteins separated on a 15% native PAGE, stained with Coomassie blue. BmPBP, recombinant protein from the silkworm moth *B. mori* as a reference; ANT20 and ANT 200, extracts from 20 and 200 female antennae; Leg 10 and Leg20, extracts from 10 and 20 midlegs as control tissues. The second lane of the gel was left empty to avoid possible contamination from the recombinant protein. B) Comparison of protein profiles from male (MA) and female antennae (FA; 600 antennae of each sex), highlighting a female-specific protein (arrow). C) Sequence alignment of *Cx. quinquefasciatus* odorant-binding protein (CquiOBP) and a product deduced from *D. melanogaster* PBPRP-3 cDNA, which encodes a protein with the highest amino acid similarity in the database. The N-terminal sequence of the mature protein, obtained by Edman degradation, is underlined. Arrowheads show the six Cys residues, a hallmark of insect OBPs. The cDNA sequence has been submitted to the GenBank data base with accession number AF468212. D) cDNA fragment (arrow) amplified with gene-specific primers by using female antenna (FA) cDNA. Under the same conditions, PCR with female leg (FL) cDNA gave only primer dimers (bottom of the gel).

antenna-specific band (Figure 1A), which had a mobility similar to the pheromone-binding protein previously identified from the silkworm moth, *Bombyx mori* (BmPBP). Comparison of extracts from mosquito male and female antennae demonstrated that the antenna-specific protein was also specific to females (Figure 1B). We then transferred the protein from native gels to polyvinyl difluoride membranes to obtain its N-terminal amino acid sequence by Edman degradation.

The sequence (DVTPRRDAEYPPPEL) was proline-rich and showed significant amino acid identity to putative odorant binding proteins from *D. melanogaster* (Pikielny et al., 1994). The same protein was not detected in legs.

Our initial approach to clone the cDNA encoding the isolated female and antenna-specific protein was based on a degenerate primer designed according to the N-terminal amino acid sequence and an oligo (dT) to anneal to the poly(A) tail. The initial product amplified by PCR (3′-RACE) was used to prepare a gene-specific primer (GSP-1) so as to obtain the full length cDNA by 5′-RACE. The cloned cDNA consisted of 708 bp, with an open reading frame (ORF) of 450 bp, a 3′-noncoding region characterized by an AATAAA signal located 15 bases upstream of a poly(A) sequence. This cDNA encodes a peptide consisting of 149 amino acids, with the first 24 residues forming the signal peptide. The signal peptide was predicted by using von Heijne's criteria for eukaryotic signal peptides, confirmed by the data for the N-terminal sequence of the mature protein, and showed the expected hydrophobic region in a Kyte-Doolitle profile.

The mature protein encoded by this cDNA consisted of 125 amino acid residues (Figure 1C), with a calculated molecular mass of 14,504 Da, and pI of 5.5. The sequence showed the hallmark of odorant-binding proteins, six Cys residues at positions 26, 53, 57, 95, 104, and 113. In addition, prediction of the secondary structure by the Chou-Fasman method indicated that the protein is helical-rich (60.8%), which is another feature of insect odorant-binding proteins.

The tissue-specificity of the female protein was further investigated by RT-PCR by using two gene-specific primers to give a 199 bp fragment of the ORF. The expected cDNA fragment was amplified with female antenna, but not with leg template (Figure 1D). Because the protein is tissue-specific and shows remarkable similarity to previously identified odorant-binding proteins from insects, we suggest that this female-specific protein from *Cx. quinquefasciatus* is an odorant-binding protein (CquiOBP).

A database search using FASTA indicated that CquiOPB shared significant sequence similarity with putative odorant-binding proteins from *D. melanogaster*, whose native proteins have not been isolated to date. One of these gene products named PBPRP-3 (pheromone-binding protein-related protein) (Pikielny et al., 1994) showed the highest score (58.6% identity) (Figure 1C). The next highest scores were from OBPs from scarab beetles (34–35% identity) (Wojtasek et al., 1999; Peng and Leal, 2001; Deyu and Leal, 2002), whereas lepidopteran OBPs (Vogt et al., 1999) showed lower amino acid identities (<29%).

Various mosquito behaviors are mediated by volatile semiochemicals. It is also known that the semiochemical-detecting machinery is involved in the perception of the synthetic repellent DEET (Leal and Uchida, 1998; Costantini et al., 2001). Therefore, odorant-binding proteins may be invaluable tools for the screening of mosquito attractants and repellents. Given that only compounds that bind to OBPs are transported to the olfactory receptors, a throughput binding assay with

recombinant OBPs would allow the screening of a large number of compounds of potential application in mosquito trapping or repellents.

Acknowledgments—This research was supported by a Specific Cooperative Agreement (No. 58-1275-1-042) with the USDA-ARS Chemicals Affecting Insect Behavior Laboratory, the University of California Systemwide Mosquito Research Fund, and through direct financial support from the department, college, and Chancellor's office at UCD. We thank Drs. Jeffrey R. Aldrich and Jerry Klun (USDA-ARS-Chemical Affecting Insect Behavior Laboratory) for their support for this project and their critique of an earlier draft version of the manuscript.

REFERENCES

COSTANTINI, C., BIRKETT, M. A., GIBSON, G., ZIESMANN, J., SAGNON, N. F., MOHAMMED, H. A., COLUZZI, M., and PICKETT, J. A. 2001. Electroantennogram and behavioural responses of the malaria vector *Anopheles gambiae* to human-specific sweat components. *Med. Vet. Entomol.* 15:259–266.

DAVIS, E. E., and BOWEN, M. F. 1994. Sensory physiological basis for attraction in mosquitoes. *J. Am. Mosq. Control Assoc.* 10:316–325.

DEYU, Z., and LEAL, W. S. 2002. Conformational isomers of insect odorant-binding proteins. *Arch. Biochem. Biophys.* 397:99–105.

LAURENCE, B. R., and PICKETT, J. A. 1982. *erythro*-6-Acetoxy-5-hexadecanolide, the major component of a mosquito oviposition attractant pheromone. *J. Chem. Soc. Chem. Commun.* 59–60.

LEAL, W. S., and UCHIDA, K. 1998. Application of GC-EAD to the determination of mosquito repellents derived from a plant, *Cymbopogon citratus*. *J. Asia-Pacific Entomol.* 1:217–221.

MICHAEL, E., BUNDY, D. A. P., and GRENFELL, B. T. 1996. Re-assessing the global prevalence and distribution of lymphatic filariasis. *Parasitology* 112:409–428.

PENG, G. H., and LEAL, W. S. 2001. Identification and cloning of a pheromone-binding protein from the oriental beetle, *Exomala orientalis*. *J. Chem. Ecol.* 27:2183–2192.

PIKIELNY, C. W., HASAN, G., ROUYER, F., and ROSBASH, M. 1994. Members of a family of *Drosophila* putative odorant-binding proteins are expressed in different subsets of olfactory hairs. *Neuron* 12:35–49.

VOGT, R. G., CALLAHAN, F. E., ROGERS, M. E., and DICKENS, J. C. 1999. Odorant binding protein diversity and distribution among the insect orders, as indicated by LAP, an OBP-related protein of the true bug *Lygus lineolaris* (Hemiptera, Heteroptera). *Chem. Senses* 24:481–495.

WOJTASEK, N., PICIMBON, J. F., and LEAL, W. S. 1999. Identification and cloning of odorant binding proteins from the scarab beetle *Phyllopertha diversa*. *Biochem. Biophys. Res. Commun.* 263:832–837.