Protein Expression and Purification 75 (2011) 165–171

Contents lists available at ScienceDirect

Protein Expression and Purification

journal homepage: www.elsevier.com/locate/yprep

Expression, purification and functional analysis of an odorant binding protein AaegOBP22 from *Aedes aegypti*

Gang Yang [a,*], Gösta Winberg [b], Hui Ren [b], Shuguang Zhang [b]

[a] Institute for Nanobiomedical Technology and Membrane Biology, Sichuan University, Keyuan 4 St., Gaopeng Avenue, Chengdu 610041, China
[b] Center for Biomedical Engineering, NE47-379, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139-4307, United States

ARTICLE INFO

Article history:
Received 14 July 2010
and in revised form 2 September 2010
Available online 7 September 2010

Keywords:
Aedes aegypti
Circular dichroism
Fluorescent probe
Odorant binding protein
Protein purification

ABSTRACT

Mosquitoes that act as disease vectors rely upon olfactory cues for host-seeking, mating, blood feeding and oviposition. To reduce the risk of infection in humans, one of the approaches focuses on mosquitoes' semiochemical system in the effort to disrupt undesirable host–insect interaction. Odorant binding proteins (OBPs) play a key role in mosquitoes' semiochemical system. Here, we report the successful expression, purification of an odorant binding protein AaegOBP22 from *Aedes aegypti* in heterologous system. Protein purification methods were set up by Strep-Tactin affinity binding and size-exclusion chromatography. Analysis by SDS–PAGE and mass spectrum revealed the protein's purity and molecular weight. Circular dichroism spectra showed the AaegOBP22 secondary structure had a pH dependent conformational change. The protein functions of AaegOBP22 were tested by fluorescent probe 1-NPN binding assays and ligands competitive binding assays. The results show AaegOBP22 proteins have characteristics of selective binding with various ligands.

Introduction

The success of host-seeking, mating, blood feeding and oviposition determine life history strategies of mosquitoes. Each of these behaviors is mediated by both internal and external factors. The most important external factor affecting mosquito behavior is olfactory cue. Many behavioral expressions of mosquitoes are mediated by olfaction [1,2]. For example, female *Anopheles gambiae* mosquitoes, which are the main vectors of malaria transmission in sub-Saharan Africa, use olfactory cues to find human hosts and avoid non-human hosts [3–6]. *Aedes aegypti* mosquitoes are carriers of dengue and yellow fever, using olfactory cues for foraging and oviposition [7,8]. To reduce the risk of infection in humans, one of the approaches focuses on the semiochemical systems of mosquitoes and other insects in the effort to disrupt undesirable host–insect interaction. Indeed, the chemical ecology of mosquitoes is now widely recognized as one area of investigation on which future vector-borne disease control strategies may depend [3,9].

Perception of volatile semiochemicals in mosquitoes is mediated, as for other insects, by chemosensory neurons segregated within specific olfactory sensilla located mainly on the antennae and maxillary palps [8,10,11]. These semiochemicals, such as pheromones, plant volatiles or animal odors are small hydrophobic molecules which enter the antennae and other sensory organs via pores and pass across the hydrophilic sensilla lymph surrounding the olfactory neuronal dendrites. The sensilla lymph containing extremely high concentrations of odorant binding proteins (OBPs)[1], including the pheromone-binding proteins (PBPs) and the so-called general odorant binding proteins (GOBPs), which solubilize and transport the odorant molecules from the porous cuticular surface of the antennal sensilla through the sensilla lymph to the G-protein-coupled odorant receptors (ORs) residing on the olfactory sensory neuron [12,13].

Considerable progress has been made in the field of olfaction with respect to mosquito–host interactions. The recent publications of *A.* aegypti OBPs and *A.* gambiae OBPs as well as ongoing sequencing projects of other important mosquito vectors offer new opportunities to advance our knowledge on mosquito olfaction [14]. *A.* gambiae and *A.* aegypti are two kinds of the most studied mosquito species. AgamOBP1 is one of OBPs found from the *A. gambiae*. By circular dichroism (CD) assays and AgamOBP1/bombykol ligand binding assays, Wogulis et al. found the conformational change of AgamOBP1 led to a significant loss of ligand affinity capacity when pH dropped from 7.0 down to 5.5 [3]. Li et al. identified the recombinant protein *A.* aegypti OBP22 could bind to a variety of chemical odors containing one or two benzene

* Corresponding author.
E-mail addresses: yanggangscu@gmail.com (G. Yang), shuguang@mit.edu (S. Zhang).

[1] *Abbrevations used:* OBPs, odorant binding proteins; PBPs, pheromone-binding proteins; GOBPs, general odorant binding proteins; ORs, odorant receptors; CD, circular dichroism; MW, molecular weight; CVs, column volumes.

1046-5928/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.pep.2010.09.004

ring structures [7]. These characteristics of selective binding of various ligands widely exist in mosquito OBPs and the OBPs of *Drosophila melanogaster,* honeybee, locusts and rat [1,15–17].

In our recent studies, we focused on developing a heterologous system for producing OBPs, and studied the functions of the AaegOBP22. In this article, we report the successful expression, purification of AaegOBP22 by the way of *Escherichia coli* extracellular secretion. Protein purification methods were set up by Strep-Tactin affinity binding and size-exclusion chromatography. Analysis by SDS–PAGE and mass spectrum revealed those protein purify and molecular weight (MW). CD spectra showed the AaegOBP22 underwent a pH dependent conformational change of secondary structure. The protein functions of AaegOBP22 were tested by fluorescent probe 1-NPN binding assays and ligands competitive binding assays. The results show AaegOBP22 proteins have characteristics of selective binding with various ligands.

Our work provides a new approach to study OBPs; it will enhance the understanding of mosquitoes' semiochemical system and develop new disease control strategies against mosquitoes. Moreover, our work will likely facilitate the design of bionic artificial nose based on nano-bio devices for a wide range of applications, from detection of infinitesimal amounts of odors, emitted from diverse diseases and environment to develop artificial organs.

Materials and methods

Reagents and buffers

All common chemicals were obtained from either Sigma (St. Louis, MO) or VWR International unless otherwise indicated. Liquid growth medium used for *E. coli* culture was Luria–Bertani (LB) medium. SDS–PAGE gels and protein standards were purchased from Invitrogen (Carlsbad, CA). Protein purification materials were purchased from GE Healthcare Life sciences (Uppsala, Sweden).

Buffer for *E. coli* culture (KPO_4 buffer): 940 ml 1 M K_2HPO_4 + 60 ml 1 M KH_2PO_4, pH 8.0. Buffers for Strep-Tactin column: (1) buffer W1: 100 mM Tris–Cl pH 9.0, 150 mM NaCl, 1 mM EDTA, 1 mM DTT; (2) buffer W2: buffer W1 + 0.2% Triton-X-114; (3) buffer E: buffer W1 + 2.5 mM desthiobiotin. Buffer for S200 gel filtration column (buffer S): 1×PBS buffer.

AaegOBP22 heterologous expression

AaegOBP22 (GenBank accession no. EAT42725) gene was selected from GenBank. The plasmids with AaegOBP22 gene were customized and ordered from GENEART (Germany). The vector backbone of the gene is pET28a (+).

The AaegOBP22 plasmids were transformed into BL21(DE3)-STAR-pLysS competent cells, then the cells were spread on LB-agar plates, followed by overnight culture at 37 °C. The colonies from LB-agar plates were selected and cultured in 5 ml of LB liquid medium, plus 50 μl of 50% glucose, overnight at 37 °C with shaking. The next morning, 1 ml of overnight culture was inoculated in 100 ml of fresh LB liquid medium, plus 1 ml of 50% glucose, and cell culture was continued at 37 °C with shaking while monitoring growth of the culture by measuring the optical density at 600 nm (OD_{600}). At OD_{600} of 0.6–0.8, 100 ml of culture was inoculated into 3 L LB, plus 30 ml of 50% glucose and 90 ml KPO_4 buffer. The cell culture was continued again at 37 °C with shaking while monitoring growth of the culture. Once OD_{600} reached 0.6 again, the temperature was decreased to 16 °C and after 20 min, the inducer was added (1 mM IPTG). The concentration was monitored every 2 h until harvested at 16 h post induction. All plates and LB liquid media used here contained 25 μg/ml of kanamycin.

The harvested media were centrifuged at 10.000 rpm at 4 °C for 1 h in Avanti J-E (Beckman); decanted supernatant; corrected pH to 9.0 by adding 1 M NaOH while stirring the supernatant; added 100 mM PMSF and 100 μg/ml ampicillin; kept at 4 °C.

AaegOBP22 protein purification

Before loading on Strep-Tactin column, the supernatant was added 0.2% TritonX-114 and filtered by using a 0.22 μm filter. The Strep-Tactin column contained 10 ml Strep-Tactin beads (IBA BioTAGnology, Germany). The supernatant was loaded on the Strep-Tactin column by a peristaltic pump at a rate of 2 ml/min at +4 °C cold room. After loading, the Strep-Tactin column was washed with 5 column volumes (CVs) of wash buffer W1, and continued with 5 CVs wash buffer W2. For eluting the target proteins, the Strep-Tactin column was connected to an Äkta Purifier HPLC system (GE Healthcare). The target proteins were eluted with buffer E over 5 CVs.

The elution fractions were tested by SDS–PAGE via Coomassie blue staining. Those containing OBPs were pooled and concentrated by using a 10 kDa MWCO filter column (Millipore, USA). To improve the purity of the protein, the concentrated proteins were subjected to size-exclusion chromatography by using a Hi-Load 16/120 Superdex 200 column (Amersham Pharmacia Biosciences). The column was preequilibrated with buffer S. After loading, the column was run with buffer S at 1 ml/min and column flowthrough was monitored via UV absorbance at 280 nm and 215 nm. Protein fractions were collected using an automated fraction collector. Peak fractions were then pooled, concentrated and subjected to SDS–PAGE test. The concentration of purified proteins was determined by a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, USA).

Mass spectrometric analysis

The mass spectrum was generated from a sample of AaegOBP22 monomer. MW measurements were made by LC–MS with MIT Koch Institute Proteomics Facility's QSTAR Elite quadrupole-time-of-flight mass spectrometer.

Circular dichroism (CD) detection

The purified protein samples came from gel filtration fractions and were concentrated to 6.3 mg/ml. In order to study the secondary structural change of AaegOBP22 in different pH environment, a small amount of the concentrated AaegOBP22 were diluted with PBS of different pH, from 5.0 to 9.0. The final AaegOBP22 concentration using for CD experiments was 0.2 mg/ml, about 12 μM of AaegOBP22 proteins. CD experiments were performed on Aviv 202 spectropolarimeter (Aviv Biomedical) with a 1 mm path length QS quartz sample cell at 25 °C. The CD spectra were recorded from 190 to 240 nm of wavelength with 1 nm resolution and 2 s of average time. PBS of pH 7.4 worked as blank to correct the baseline. Results were expressed as the molar mean residue ellipticity (θ) at a given wavelength.

Fluorescent probe binding assays

Fluorescent probe binding experiments were performed with 2 μM AaegOBP22 solution in 50 mM PBS, pH 7.4. The fluorescent probe 1-NPN was purchased from Sigma–Aldrich (USA). The probe was dissolved in 10% v/v ETOH as 1 mM stock solution. To measure the affinity of the fluorescent probe 1-NPN to AaegOBP22, the 2 μM AaegOBP22 solution was titrated with aliquots of 1 mM 1-NPN solution to final concentrations of 2–16 μM 1-NPN. Spectra were recorded at 25 °C using a FluoroMax-3 spectrofluorometer (Jobin