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Insights into structural features determining odorant affinities to honey bee odorant binding protein 14

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ABSTRACT

Molecular interactions between odorants and odorant binding proteins (OBPs) are of major importance for understanding the principles of selectivity of OBPs towards the wide range of semiochemicals. It is largely unknown on a structural basis, how an OBP binds and discriminates between odorant molecules. Here we examine this aspect in greater detail by comparing the C-minus OBP14 of the honey bee (*Apis mellifera* L.) to a mutant form of the protein that comprises the third disulfide bond lacking in C-minus OBPs. Affinities of structurally analogous odorants featuring an aromatic phenol group with different side chains were assessed based on changes of the thermal stability of the protein upon odorant binding monitored by circular dichroism spectroscopy. Our results indicate a tendency that odorants show higher affinity to the wild-type OBP suggesting that the introduced rigidity in the mutant protein has a negative effect on odorant binding. Furthermore, we show that OBP14 stability is very sensitive to the position and type of functional groups in the odorant.

1. Introduction

Odorant binding proteins (OBPs) attracted increasing attention in recent years due to their potential application as biosensing elements for the fabrication of odorant sensors based on the olfactory system [1–4]. Applications are diverse and include disease diagnostics [5], food safety [6], and environmental monitoring [7]. These biomimetic sensor platforms potentially provide higher sensitivity combined with lower detection limits and faster response time compared to odorant sensors based on metal oxides and conducting polymers [8–10].

OBPs are abundant small proteins (~13–16 kDa) found in the olfactory epithelium of vertebrates and the sensillar lymph of insects [11]. The functional role of OBPs in olfaction is not fully resolved yet. However, high concentrations (10 mM) of OBPs in olfactory dendrites and the relatively high number of OBPs in the genome indicate important contributions [12,13]. A meanwhile widely accepted hypothesis describes OBPs as a carrier for hydrophobic odorant molecules through the sensillar lymph to the membrane which holds the odorant receptor cells [12,14].

The focus of this work is OBP14 of the honey bee (*Apis mellifera* L.). Investigation of the olfactory system in honey bees is of particular interest due to the high complexity of the chemical language used by these social insects to communicate among the members of the bee hive [15]. The genome of the honey bee comprises 21 OBPs [16], 13 of which are classified as classic OBPs (OBP1–13) and seven as C-minus OBPs with four Cys residues (OBP15–21). OBP14, also a member of the C-minus class, is unique, featuring five cysteines. It has been identified in different tissues of adult bees, as well as in larvae [17]. OBP14 exhibits 119 amino acid residues with a molecular weight of 13.5 kDa [18]. Typical for insect OBPs, its three dimensional structure predominantly consists of α-helical domains arranged in a very compact and stable structure, as depicted in Fig. 1A. Featuring five cysteines, OBP14 exhibits two disulfide bonds between residues 17(α1)–49(α3) and 88(α5)–106(α6) as well as an unpaired cysteine at position 47(α3) [18]. For investigation of the functional implications arising from structural differences between classic and C-minus OBPs, a double mutant Q44C-H97C of OBP14 was employed in this study, which

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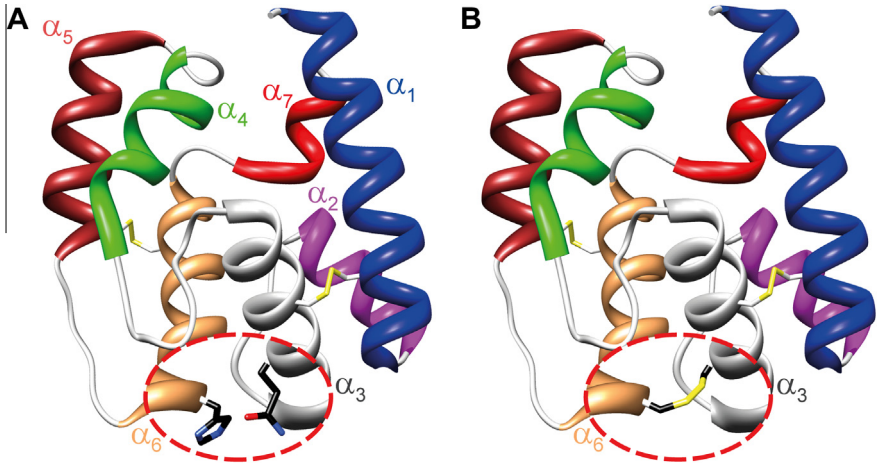

Fig. 1. Three dimensional model of (A) wild-type OBP14 and (B) mutant OBP14 featuring an additional disulfide bond between α3 and α6. OBP14 natively possesses two disulfide bonds between α1–α3 and α5–α6, respectively, thus being classified as a C-minus OBP.

comprises the third disulfide bond present in classical OBPs (see Fig. 1B) [18].

Ligand-binding characteristics and affinities of a wide range of odorants to OBPs of various species have been the subject of intensive research [19–21]. Typically, fluorescence binding studies are employed to indirectly determine the affinity of an odorant relative to a fluorescence reporter molecule [17,18,22,23]. Most recently, our lab presented a method of estimating odorant affinities to OBPs by monitoring the changes of thermal stability of the protein upon odorant binding by circular dichroism (CD) spectroscopy. This approach has been successfully applied to OBP14 and has been validated by infrared (IR) spectroscopy [24]. By evaluation of the different transition temperatures of geraniol and eugenol, it was possible to distinguish between the affinities of the two ligands. CD is a convenient method for studying the structure of proteins in solution and is particularly applicable to monitor dynamic changes in the secondary structure triggered by an external perturbation such as a temperature increase [25].

Increased protein stability upon ligand binding has been observed for a wide variety of biological systems [26–28]. Weak non-covalent forces such as hydrogen bonds as well as electrostatic, hydrophobic and aromatic interactions have been recognized to play a significant role in increasing the structural stability of the protein–ligand complexes [29–31].

In this work, we systematically analyze and evaluate structural parameters that influence an odorant's affinity to OBP14. So far, this has only been accomplished for odorant receptors [32,33]. However, with the growing interest in OBPs and their crucial role in olfaction, structural properties of their binding cavity are the consequential target of future investigations. To address this question, we employed CD spectroscopy to compare the effect of ligand binding on the thermal stability of wild-type and mutant OBP14 and correlate the increase of stability with odorant affinity. The tested odorants include eugenol and its structural analogues, which belong to the family of phenyl propanoids, a group of compounds known for their role as semiochemicals for many insects [34]. Comparison of the wild-type and a mutant form of OBP14 reveals the impact of protein flexibility on the OBP's ability to adapt its binding cavity to fit different odorants with varying functional groups.

2. Materials and methods

2.1. Materials

Eugenol (4-prop-2-enyl-2-methoxyphenol, 99%), methyl eugenol (4-allyl-1,2-dimethoxybenzene, 98%), 4-vinylguaiacol (2-methoxy-4-vinylphenol, 98%), homovanillic acid (2-(4-hydroxy-3-methoxy-phenyl)acetic acid, 98%), coniferyl aldehyde (3-(4-hydroxy-3-methoxyphenyl)prop-2-enal, 98%), coniferyl alcohol (4-(3-hydroxy-1-propenyl)-2-methoxyphenol, 98%), isoeugenol (2-methoxy-4-(prop-1-en-1-yl)phenol, 98%), dihydroeugenol (2-methoxy-4-propylphenol, 99%), 3,4-dimethoxystyrene (techn.) were provided by Sigma–Aldrich (Steinheim, Germany).

2.2. Expression and purification of OBP14

Expression of recombinant proteins was done as described in Spinelli et al. [18]. Bacterial expression was performed along with established protocols [17,35] and purification was accomplished using conventional chromatographic techniques [36,37]. The purity of the protein was checked by SDS–PAGE.

2.3. Circular dichroism

Far UV (260–195 nm) CD measurements were carried out using an Applied Photophysics Chirascan plus spectrophotometer (Leatherhead, Surrey, United Kingdom) equipped with a temperature control unit (Quantum TC125) in a 1 mm quartz cell at 1 nm resolution. Protein solutions (0.5 mg/mL; 41.8 μM) were prepared in phosphate buffer (140 mM NaCl, 3 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 8). For static measurements, ten spectra with the acquisition time of 0.5 s were taken at room temperature and the results were averaged. For measurement of OBP14 in the presence of odorants, the protein was incubated in solution with 200 μM odorant for 1 h. For temperature-controlled experiments, two acquisition techniques were employed. In spectra-kinetic mode, spectra were taken in the range of 20–90 °C (ΔT = 5 °C) with an acquisition time of 0.2 s after an equilibration time of 45 s at each temperature step. In the kinetic mode, the ellipticity was recorded at a fixed wavelength of 222 nm with an acquisition time of 0.5 s.

2.4. MD simulations

MD simulations were performed using the GROMOS11 package for biomolecular simulations [38] and the GROMOS force field 54A8 [39] starting from the native and mutant OBP14 crystal structures (PDB ID: 3S0A and 3S0G) [18]. Three 50-ns simulations each were performed at 300, 340, 360, 370, and 400 K. Detailed simulation settings are provided in the Supporting Information.