Insights on pH-dependent conformational changes of mosquito odorant binding proteins by molecular dynamics simulations

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Chemical recognition plays an important role for the survival and reproduction of many insect species. Odorant binding proteins (OBPs) are the primary components of the insect olfactory mechanism and have been documented to play an important role in the host-seeking mechanism of mosquitoes. They are "transport proteins" believed to transport odorant molecules from the external environment to their respective membrane targets, the olfactory receptors. The mechanism by which this transport occurs in mosquitoes remains a conundrum in this field. Nevertheless, OBPs have proved to be amenable to conformational changes mediated by a pH change in other insect species. In this paper, the effect of pH on the conformational flexibility of mosquito OBPs is assessed computationally using molecular dynamics simulations of a mosquito OBP CquiOBP1 bound to its pheromone 3OG (PDB ID: 3OGN). Conformational twist of a loop, driven by a set of well-characterized changes in intramolecular interactions of the loop, is demonstrated. The concomitant (i) closure of what is believed to be the entrance of the binding pocket, (ii) expansion of what could be an exit site, and (iii) migration of the ligand towards this putative exit site provide preliminary insights into the mechanism of ligand binding and release of these proteins in mosquitoes. The correlation of our results with previous experimental observations based on NMR studies help us provide a cardinal illustration on one of the probable dynamics and mechanism by which certain mosquito OBPs could deliver their ligand to their membrane-bound receptors at specific pH conditions.

Keywords: odorant binding protein; mosquito; pH; dynamics; ligand binding

Introduction

Odorant binding proteins (OBPs) are an important family of proteins involved in the olfactory mechanism, the primary mechanism used by the mosquitoes for the identification of a human host. With the current knowledge available on OBPs of insects, it is now strongly believed that OBPs serve as passive transporters involved in importing the odorant molecule from the sensillium lymph to the neuronal membrane, where they are presented to the olfactory receptors for a further downstream signaling process. For an OBP to function as a carrier and for it to play additional putative roles in odor discrimination, receptor activation and odorant deactivation, its uptake/release mechanism needs to be individually tuned (Steinbrecht, 1998). OBPs are represented as rough conical structures with six helices, four (α1, α2, α3, α4) of which converge to form the hydrophobic binding pocket capped by helix α3 by attaching it to the flanking helices (α5 and α6) resulting in a rigid compact structure. The change in pH, as observed from the sensillium lymph to the vicinity of the membrane, has been proved to trigger conformational changes in the structure of several lepidopteron OBPs (Damberger et al., 2000; Leal, Chen, & Erickson, 2005; Leal et al., 2009; Wojtasek & Leal, 1999). Such conformational changes have been described to directly have an effect on the ligand binding and release mechanism of these OBPs.

The ligand binding and release mechanism of mosquito OBPs, however remains unexplored. Nevertheless, several hypotheses have been proposed on the possible role of pH on the conformational stability of the mosquito OBPs. Evidence for a change in the tertiary structure of
AegOBP1 from *Aedes aegypti* at different pH conditions was observed by the analysis of spectral changes using circular dichroism (Leite et al., 2009). Further hypothesis on the structural segments involved in such conformational changes was described based on observations made on the structure of CquiOBP1 an OBP from *Culex* (Mao et al., 2010). The possible role of the C-terminal loop in the ligand binding and release mechanism was described. The C-terminal end of the mosquito OBPs, which in general in short as observed in the case of LUSH *Drosophila* OBP (Kruse, Zhao, Smith, & Jones, 2003; Laughlin, Ha, Jones, & Smith, 2008; Thode, Kruse, Nix, & Jones, 2008; Xu, Atkinson, Jones, & Smith, 2005), forms a wall of the binding pocket stabilized by hydrogen bonds formed between the terminal residue and residues in helix α1 and helix α3. These hydrogen bonds were proposed to form a “pH sensing triad” which could be disrupted at lower pH conditions and displace the C-terminal end from the binding pocket. The release of the C-terminus was thus expected to play an important role in the ligand binding and release mechanism. It was also demonstrated that the residues in helix α5 of the same protein exhibit exchange-broadened NMR resonances, suggesting a conformational exchange in this region which functions as a gate for the binding of mosquito oviposition pheromone (MOP) inside the protein (Mao et al., 2010).

Based on these assumptions of a pH-dependent conformational change in the case of mosquito OBPs, we demonstrate the effect of pH on the conformational flexibility of CquiOBP1 from *Culex* starting from its crystal structure (PDB ID: 3OGN, where the protein is in complex with an oviposition pheromone MOP/3OG (C18 H32 O4)) using molecular dynamics and essential dynamics analysis. We provide additional evidence that OBPs in mosquitoes indeed undergo a pH-dependent conformational change. However, in contrast to the previously proposed hypothesis that suggests the role of the C-terminal end in the ligand binding and release mechanism, we propose that the C-terminal loop is not affected by a change in pH. Alternately, a loop between helix α3 and helix α4 is found to undergo a conformational twist in response to the change in pH. This change in the conformational state of the loop affects some of the interaction patterns between helix α4 and helix α5, which in turn facilitates the release of the ligand. The described mechanism, in conjugation with earlier experimental observations based on NMR studies on the CquiOBP1-MOP complex at pH 7.0 described in Mao et al. (2010), provides the very first insight into the probable pH-dependent mechanism of ligand binding and release in the case of mosquito OBPs.

**Methodology**

GROMACS 4.5.3 (Van Der Spoel et al., 2005) molecular dynamics package was used for the simulations. Simulations were carried out on the *Culex* OBP (CquiOBP1) crystal structure PDB ID: 3OGN with the ligand at two different pH conditions pH = 8.2 (native pH) and pH = 4.0 (low pH). The pH change was mimicked by the protonation of histidine residues based on the pka values predicted by PROPKA (Li, Robertson, & Jensen, 2005). Eight of the nine histidine residues in the structure were protonated at low pH. His111 was not protonated, since it was found more buried in the structure and it had a low pKa value of 2.93. The OPLS-AA force field (Jorgensen & Maxwell, 1996) was used for the protein, as well as for the ligand MOP/3OG (C18 H32 O4) (1S)-1-[(2R)-6-oxotetrahydro-2H-pyran-2-yl] undecyl acetate were taken from Price and Ostrovsky (2001). Same for the ester, aromatic ester ring like groups of the ligand (Price & Ostrovsky, 2001), the aliphatic side chain, and methyl groups (Jorgensen & Maxwell, 1996), the OPLS-AA force field was used. The starting structures of every simulation were solvated in cubic periodic water boxes with a 1.4 nm solute-wall distance. The system was energy minimized twice before and after the addition of ions. The native pH system was neutralized using seven Na+ atoms and the low pH system was neutralized using one Cl− ion. The size of the system was approximately 28,500 atoms. The system was equilibrated for 100 ps with position restraints prior to the 50 ns production run. Two femtosecond time step was used for integration of the equations of motion. Solute and solvent with the ions were weakly coupled to a reference temperature bath at 300 K with a coupling constant T of .1 ps (Berendsen, Postma, van Gunsteren, DiNola, & Haak, 1984). The pressure was maintained by weakly coupling the system to an external pressure bath at one atmosphere with a coupling constant of 1.0 ps and an isothermal compressibility of 4.5 × 10−5 bar−1 (Berendsen et al., 1984). Short-range coulombic and van der Waals cut-off of 1.0 nm were used and long-range electrostatics were treated using Particle-Mesh-Ewald with an interpolation order of 4 and a Fourier spacing of .12 nm (Darden, York, & Pedersen, 1993; Essmann et al., 1995). The same experimental setup was duplicated using different initial velocities each 50 ns in length for each of the different starting structures in order to improve conformational sampling. Ten simulation systems were setup: six at high pH simulations and four at low pH simulations.

**Results**

**pH-dependent change on the conformational stability**

The available crystal structure of CquiOBP1-3OG protein–pheromone complex was subjected to MD simulations at two different pH condition high pH (8.2) and low pH (4.0). The conformational stability of the two systems was assessed by the drift of the protein from the initial structure using RMSD of Cα atoms.
Throughout each simulation, it was observed that the overall conformation of the protein remained close to the crystal structure. However, it was observed in general that the systems at low pH (pH 4.0) showed higher variations in terms of side-chain flexibility compared to the systems at native pH (pH 8.2), as measured by the RMS fluctuations of individual residues. Increased flexibility was observed for residues from 63 to 74 (which is the loop connecting helix α3 and helix α4) and was featured in the case of low pH simulations. Interestingly, in addition to this region, large fluctuations were also observed for residues from 77 to 124 which involve residues from helix α4, helix α5, and helix α6 specific only to the low pH simulation (Figure 1). This proved to be a critical observation of our analysis which is further detailed below.

*pH* sensing triad

We confirm that the C-terminal end of Cquiobp1 is located inside its binding pocket and is stabilized by hydrogen bonds between residues in the terminal region, and the residues from helix α1 and helix α3. Similar hydrogen bond triad in the C-terminus of the protein is also described in the structures of AgamOBP1 and AaegOBP1. As hypothesized by Mao et al. (2010), the disruption of this hydrogen bond network at low pH was expected to destabilize the C-terminal loop and displace the C-terminal from the central cavity facilitating the release of the ligand and hence serving as a pH sensing lock. These hydrogen bond interactions (Mao et al., 2010) that would clamp the C-terminus were hence scrutinized throughout the low pH simulations. The distances of the carboxylate group of Val125 with the hydroxyl group of Tyr54 and δ-nitrogen of His23 were monitored throughout the low pH simulations (Figure 2(A)). However, interestingly, it was observed that the distance between His23 and Val125 did not vary from the typical hydrogen bond distance range in any of the low pH simulations, suggesting a strong interaction between these two residues even at low pH conditions. The distance between Tyr54 and Val125 was found to be more fluctuating (Figure 2(B)), suggesting it to be a rather weak bonding. However, this does not affect the position of the C-terminal end of the protein which is held strongly by the other hydrogen bond (Figure 2(C) and (D)).

Loop between helix 3 and 4

Close examination of the other regions of the protein with high RMS fluctuations revealed that the loop residues from 63 to 74 between helix α3 and helix α4 showed considerable flexibility only in the case of the low pH simulations. This loop, hereafter named loop3, was in fact previously described to undergo a pH-dependent conformational change in the case of *Bombbyx mori* (BmorPBP1) (Gräter, de Groot, Jiang, & Grubmüller, 2006; Sandler, Nikonova, Leal, & Clardy, 2000) and *Apis mellifera* (ApolPBP1) (Damberger, Ishida, Leal, & Wüthrich, 2007). It was interesting to note that this loop3 indeed adopts a new conformation during the course of the low pH simulations. The observed change in the conformation of the loop3 was mediated by a change in a confined set of intramolecular interactions of the loop with the helices. This loop3 interacts with helix α6 via a salt bridge between Asp70 and Lys106 and a hydrogen bond between Lys75 and Val65 with helix α4. In order to measure the stability of these hydrogen bond interactions (Mao et al., 2010) that would clamp the C-terminus were hence scrutinized throughout the low pH simulations. The distances of the carboxylate group of Val125 with the hydroxyl group of Tyr54 and δ-nitrogen of His23 were monitored throughout the low pH simulations (Figure 2(A)). However, interestingly, it was observed that the distance between His23 and Val125 did not vary from the typical hydrogen bond distance range in any of the low pH simulations, suggesting a strong interaction between these two residues even at low pH conditions. The distance between Tyr54 and Val125 was found to be more fluctuating (Figure 2(B)), suggesting it to be a rather weak bonding. However, this does not affect the position of the C-terminal end of the protein which is held strongly by the other hydrogen bond (Figure 2(C) and (D)).

**Figure 1.** RMS fluctuation plots of classic OBP in the ligand-bound form for the Cα atoms of every residue in native pH (left) and at a low pH of 4.0 (right) for different simulation systems as an average over 50 ns. The different duplicates for each simulation system are represented in different colors. The residues which undergo higher fluctuations, other than N-terminal region, are boxed. Ligand-bound OBP show higher fluctuations at pH 4.0.
interactions, we monitored the distances between the NZ and CG atoms of Lys106 and Asp70 throughout the simulation at the two pH conditions. It was observed that the distance between these residues increased in the case of the low pH simulation, but remained unaffected in the case of high pH simulation experiments. This indicates a loss of interaction between Asp70 and Lys106 only in the low pH simulations. Similarly, the interaction of the loop3 with helix α4 was monitored by measuring the distance between NZ and C atoms of Lys75 and Val65. The distance between these residues also increased in the case of low pH systems, while the distance in the case of high pH remained within hydrogen bonding distance. This observation indicates a loss of this interaction between Lys75 and Val65 only in the low pH environment (Figure 3).

Compensating interactions of the loop3 with helix α3

Intriguingly, we observed that the interactions lost by the loop3 with helix α4 and helix α6 are compensated by a new salt bridge that is formed between Asp67 of the loop3 and His60 on helix α3 in the case of low pH simulations. The distance between CG and ND1 atoms of these residues was monitored throughout the high and low pH simulations. The distance between these atoms reduced only in the case of low pH systems compensating the loss of interaction between Asp70 and Lys106. Thus, in the case of low pH simulations, the loop3 loses its interaction with helix α4 and helix α6 and forms a new interaction with helix α3 which in turn induces a change in the conformational state of the loop (Figure 3).

Change in interaction patterns of helix α4 and helix α5

As mentioned previously in the paper, we observed that the residues in helix α4 and helix α5 of the protein also indicate high RMS fluctuations in the case of the low pH systems (Figure 1). A detailed analysis on this region of the protein indicated changes in the interaction pattern of certain residues which is again specific only to the low pH simulations. During the course of simulations, Asp70 of loop3, which was previously interacting with helix α6 via a salt bridge, now forms a new interaction with His72 (measured as a distance between ε-nitrogen of His72 and the carboxylate carbon of Asp70). This
triggers a correlated increase in the distance between His72 and Glu74 and is represented here as a measure of distance between HE1 and OE atoms of His72 and Glu74, respectively. This is followed by a new interaction of the δ-nitrogen of His77 with the carboxylate group of Glu74. As a consequence, the interaction between the main-chain oxygen of His85 and ε-nitrogen of His77 is disrupted (Figure 4). The distances of the above stated interactions were also monitored for the high pH systems. It is very intriguing that these changes in the interaction patterns are indeed highly specific to the low pH systems.

**Binding pocket and mobility analysis of MOP**

In order to get views on the mobility of the ligand at the two different pH conditions, we observed the RMS fluctuations of the ligand at two different pH conditions. It was clearly observed that the ligand was more flexible at the low pH simulations than the high pH simulations (Supplementary Figure 4), which is in good agreement with the higher fluctuations of the C-ter region of the protein (see boxed residues in right panel of Figure 1). Accordingly, in case of the low pH simulations, we observed a partial unbinding of MOP from the entrance of the hydrophobic tunnel encompassed by helix α4 and α5. The entrance of the hydrophobic tunnel found between helix α4 and α5 tends to close upon the partial unbinding of the ligand. Interestingly, the partially unbound ligand moves towards the opening located at the convergence of helix α5, helix α1, helix α3, and the loop3 that undergoes a conformational change (Supplementary movie 1). Upon tracking the various interactions of MOP at the end of simulations, an extension of the hydrophobic tunnel was observed. Residues that mainly contributed towards this extension were Ala18, His23, Leu58, Phe59, Ala62, Val64, Lys75, Met84, Met89, Leu96, Val125, and Leu124 but not the ligand binding residues at the initial state of the ligand in the crystal structure. This clear representation of a hydrophobic tunnel, at the convergence of helix α3, helix α4, helix α1, and loop3, suggests that this could be the exit site for the ligand.
Essential dynamics analysis

To analyze the effect of the change in interaction patterns on the protein motions, we extended our analysis using the essential dynamics analysis or principal component analysis. The loss of a salt bridge between the terminal residue of the loop3 and helix $\alpha_6$ and the loss of a hydrogen bond between Val65 and Lys75 causes a conformational drift of the loop3 leading to an anti-clockwise flip of the loop, with respect to helix $\alpha_3$ and helix $\alpha_4$. This loss of interaction makes the side chains of Asp67 and Asp70 available for new salt bridges with His60 in helix $\alpha_3$ and His72 in helix $\alpha_4$, respectively. Likewise, the interaction between Asp67 and His60 tilts the loop toward helix $\alpha_3$. Thus, the conformational change of loop3 is mediated by a correlated change in the interaction pattern, mainly involving the charged residues in the loop. With the loss of the hydrogen bond between Lys75 and Val65, Lys75 is made accessible for interaction with the ligand. This also results in a noticeable change in the orientation of helix $\alpha_4$, increasing the distance between helix $\alpha_1$ and helix $\alpha_4$. The interaction of His72 of helix $\alpha_4$ with Asp67 increases the distance between His72 and Glu74. Instead, Glu74 is engaged in a salt bridge with His77 which causes the closure of the entrance of the binding pocket (Figure 5). The observations made based on the essential dynamics analysis can be correlated to the change in the interaction patterns described above (Supplementary movie 2).

Discussion

CquiOBP1 undergoes a pH-dependent conformational change

Globular proteins, in general, are capable of reacting to changes in environmental conditions such as temperature, pH, and ligand concentration. They can also change
their conformation that is facilitated by a number of interdependent cooperative interactions embedded in the rather complicated steric arrangement of their polypeptide chain. OBPs have been previously described to undergo conformational changes, mediated by both pH change and ligand binding, for their primary roles in olfaction. The pH change at the vicinity of the dendritic membrane was described to induce necessary conformational changes in the protein and release of the ligand which then directly activates the corresponding odorant receptors. Alternatively, it has also been described that a few OBPs are capable of activating the receptor in complex with the ligand without the release of the ligand (Bette, Breer, & Krieger, 2002; Campanacci et al., 2001; Honson, Johnson, Oliver, Prestwich, & Plettner, 2003; Kruse et al., 2003; Mohl, Breer, & Krieger, 2002; Xu et al., 2005). This is presumed to be facilitated by conformational changes of the protein caused by ligand binding, which are further recognized by their receptors (Laughlin et al., 2008). However, this model has been challenged in a very recent study where transgenically expressed mutants of LUSH OBP known to reproduce the same conformational changes when bound to the ligand, fails to affect neither basal nor pheromone-induced activity in Drosophila melanogaster (Gomez-Diaz, Reina, Cambillau, & Benton, 2013). Their experimental data clearly suggest direct activation of the odorant receptor by the ligand and hence support the model by which the ligand has to be released from OBP before it can activate the receptor. Nevertheless, experimental evidence for a pH-dependent conformational change in OBP is well-documented. It has been described in AgamOBP1, and AaegOBP1 using CD spectra associated with loss of affinity for ligand in the case of AgamOBP1 (Wogulis, Morgan, Ishida, Leal, & Wilson, 2006) and change in the near-UV CD spectra indicating a change in the tertiary structure in the case of AaegOBP1 (Leite et al., 2009). It has also been very recently described that the ligand-induced conformational ordering can play a key role in regulating the heteromeric interactions between OBPs using the structure of AgamOBP4 (Wogulis et al., 2006). CquiOBP1, which is an ortholog of AgamOBP1 and AaegOBP1, was also predicted to undergo a pH-dependent conformational change. With the assumption that CquiOBP1 undergoes a pH-dependent conformational change, we simulated the protein with the ligand at different pH conditions. The change in pH was mimicked by changing the protonation states of histidine residues of CquiOBP1. The pH change was found to have a minimal effect on the overall stability of the protein with intact secondary structure, corresponding to the results as observed for AaegOBP1 using CD spectra (Leite et al., 2009). However, the results indicated higher RMS fluctuations observed in certain regions of the protein specific to low pH simulations, which is completely absent in the high pH systems. This strongly suggests that CquiOBP1, similar to AgamOBP1 and AaegOBP1, is prone to undergo conformational changes in response to a change in pH without loss of secondary structure.
Does the previously hypothesized “pH sensing triad” of the C-terminal carboxylate contribute to conformational changes seen in the case of low pH simulations?

The C-terminal end of BmorOBP1 (OBP from *B. mori*) is described to play an important role, undergoing a significant conformational change at low pH. The C-terminal end which instead of being outside the binding pocket, folds itself into a helical structure inside the binding pocket of the ligand (Damberger et al., 2000; Gräter et al., 2006; Sandler et al., 2000; Wogulis et al., 2006). However, in the case of few OBPs like LUSH, AgamOBP1, AaegOBP1, and CquiOBP1, the C-terminal end is too short to form a helix that will occupy the binding pocket (Kruse et al., 2003; Leite et al., 2009; Mao et al., 2010; Wogulis et al., 2006). It was hypothesized that this hydrogen bond triad could be a pH-sensing triad, which, upon contact with a low pH environment could disrupt, releasing the C-terminal end from the central cavity. In our simulation, a close examination of the distances of the atoms involved in the triad strikingly showed that the hydrogen bond between Val125 and the hydroxyl group of Tyr54 and δ-nitrogen of His23 and few other interactions (Leite et al., 2009; Mao et al., 2010; Wogulis et al., 2006). This was supported by the fact that the crystal structure of AgamOBP4, crystallized at a lower pH of 6.8, indeed showed the C-terminus to be a part of the binding pocket (Davrazou, Dong, Murphy, Johnson, & Jones, 2011). Recent data of Rusconi et al. (2012) on AgamOBP1 found that mutations of some of the residues (F123T, Y122F, Y122A, L124T) of the C-terminal have a great influence on the conformational stability of the protein and ligand binding. CquiOBP1 which is an ortholog of AgamOBP1 retains these residues (Supplementary Figure 2), suggesting a similar role of these residues on its structure and function. The results clearly indicate that the C-terminal end indeed plays a significant role in maintaining the conformation of the protein and in ligand binding. This supports our result of the C-terminal end, not being involved in conformational changes that could drive the release of the ligand and opens up the possibility of having other possible regions of the protein taking up this role.

Loop3 of CquiOBP1 undergoes a major conformational change

In addition to the C-terminal end, a histidine-rich loop3 between helix α3 and helix α4 has been implicated in ligand binding and is described to function as a flexible lid. Loop3 is observed to adopt different conformational states in the structures of OBPs from *B. mori* and *Antheraea polyphemus* pheromone binding proteins (Damberger et al., 2007; Gräter et al., 2006; Sandler et al., 2000). It was noticed that this loop in CquiOBP1 bears a number of charged residues and adopts a conformational shift in the case of low pH simulations. At high pH conditions, this loop3 interacts with helix α6 and helix α4, but in case of low pH conditions adopts a new conformation. During our simulations, this loop undergoes an anti-clockwise rotation and interacts with helix α3, losing its previous interactions with helix α6 and helix α4 (Figure 3(b)). This observed rotation was seen in all the replicates of the simulation experiments, which is confirmed by the distance measurements as observed in Figure 3(a). This change in interactions between the helical segments of the protein and the loop3 is facilitated by change in the interaction patterns of two salt bridges and two hydrogen bonds. The loss of a salt bridge between helix α6 and the loop (Asp70–Lys106) in the case of low pH simulations is compensated by a new salt bridge between the loop and helix α3 (Asp67–His60). The hydrogen bond between the loop and helix α4 (Lys75–Val65) is replaced by another hydrogen bond between the same parts of the protein, but involving alternate residues (Asp70 and His72). Hence, we hypothesize that in CquiOBP1, this loop3 between helix α4 and helix α5 is directly affected by a change in the pH rather than the C-terminal end of the protein. This change in the conformational state of the loop3 can play important functional roles possibly providing new insights into the ligand binding and release mechanisms of CquiOBP1. It is also interesting to note that this particular loop retains a number of conserved charged residues among the classic OBPs in the mosquito genome (Supplementary Figure 3), further suggesting that this is a functional loop in other classic OBP members with a short C-terminal end.

Concerted change in interaction patterns following the conformational change of the loop3

It was interesting to note that the new conformational state adopted by the loop3, observed during our simulations, was followed by coordinated sequential changes in the interaction pattern of certain residues in helix α4 and helix α5, which in turn, causes a change in the surface area of the protein. This change in interaction patterns accounts for the high RMS fluctuations observed initially.
during the low pH simulations. The residues in helix α4 and α5 in CquiOBP1 form a part of the hydrophobic tunnel involving the binding of MOP. NMR study on the CquiOBP1-MOP complex at pH 7.0 described in Mao et al. (2010) indicated that a long stretch of amino acid residues in helix α5 exhibited exchange-broadened NMR resonances suggesting that this region may undergo conformational changes. It was further proposed that this conformational fluctuation in helix α5 may function as a gate to create an opening to permit the entrance of the binding pocket. The current results support the previously assumed hypothesis involving conformational fluctuations of the helix α5 and the observed change in the surface of the protein. The current analysis suggests that these conformational fluctuations are preceded by a change in the conformational state of the loop3 between helix α3 and helix α4. This is supported by the essential dynamics analysis which indicates correlated movements of these regions.

Exit site of the ligand

Ruling out the option of the unbinding of the C-terminal end from the binding pocket for the release of the ligand, at least in the case of CquiOBP1, the partial unbinding and movement of MOP towards the opening at the convergence of helix α1, helix α3, helix α4, and loop3 stimulate the idea of this being a possible exit route of the ligand. This can be a coordinated effect, corresponding to a change in the conformational state, which causes the movement of helix α4 increasing the distance between helix α1 and helix α4. However, a complete unbinding of MOP was not observed in the simulation. If unbinding should occur, it would require longer simulations. This opening has also been described in AgamOBP4 crystallized at a rather low pH 6.5 and it is described to be the binding site for AgamOBP1 resulting in the formation of a dimer (Davrazou et al., 2011). This leaves us with a speculation whether MOP will be released at this exit site or if ligand-induced conformational changes could occur at this end of the protein inducing the binding of other OBPs. It would be interesting to perform further extended simulations to understand the complete unbinding of the ligand. If unbinding could be observed in longer simulations at low pH and not at higher pH, this would strongly be in favor of the recently proposed model by which the ligand has to be released from OBP in order to activate odorant receptors (Gomez-Diaz, Reina, Camillau, & Benton, 2013).

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Supplementary material

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